A Role for MAP Kinase Signaling in Behavioral Models of Depression and Antidepressant Treatment

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Background: Brain-derived neurotrophic factor (BDNF) is upregulated in the hippocampus by antidepressant treatments, and centrally administered BDNF can produce antidepressant-like effects in rodent behavioral models of depression. BDNF-regulated signaling pathways are thus potential targets for investigation of antidepressant mechanisms.

Methods: We examined the effects of inhibition of MAPK kinase (MEK) in mouse behavioral models for depression including interactions with effects of antidepressant drugs. We also assessed the behavioral consequences of a heterozygous gene deletion for BDNF combined with MEK inhibition or stress.

Results: Acute administration of the MEK inhibitor PD184161 produced depressive-like behavior. PD184161 blocked the antidepressant-like effects of desipramine and sertraline in the forced swim test and blocked the effects of desipramine in the tail suspension test. Heterozygous deletion of BDNF alone did not influence behavior in the forced swim test but resulted in a depressive phenotype when combined with a low-dose MEK inhibitor or stress exposure.

Conclusions: We demonstrate that acute blockade of MAPK signaling produces a depressive-like phenotype and blocks behavioral actions of antidepressants. We also demonstrate in BDNF heterozygous knockout mice an example of how a defined genetic alteration can confer vulnerability to a pharmacologic or environmental challenge resulting in a depressive behavioral phenotype.

Key Words: BDNF, ERK, forced swim, learned helplessness, mouse, tail suspension

Despite progress in studies of the etiology and treatment of depression, a clear understanding of the mechanisms critical to the effectiveness of existing antidepressant drugs and the identification of potentially novel antidepressant mechanisms are still lacking. Antidepressant drugs are known to acutely increase synaptic concentrations of monoamines, but clinical efficacy requires several weeks of continuous treatment, suggesting that time-dependent neural adaptations, perhaps induced by acute synaptic actions, are important to therapeutic efficacy (Duman et al. 1997; Manji et al. 2001; Nestler et al. 2002). Work from this and other labs has investigated the regulation of neurotrophin expression and neurotrophic processes as candidate drug-regulated time-dependent processes that could mediate the delayed functional consequences of antidepressant treatment.

Neurotrophins mediate the differentiation and survival of neurons during development and they play a role in the regulation of survival and the modulation of synaptic transmission and plasticity in adult neurons (Henderson et al. 1996; Schuman 1999; Thoenen 1995; Tucker et al. 2001). Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor family that is implicated in depression and in antidepressant mechanisms. Expression of BDNF and its receptor trkB are increased in rat hippocampus by repeated administration of antidepressant drugs as well as by electroconvulsive seizure (Nibuuya et al. 1995; Russo-Neustadt et al. 2000). The time-dependence of this effect is consistent with the time course of clinical effectiveness of antidepressant drugs. Furthermore, BDNF is increased in postmortem brain of human subjects who had been treated with antidepressants (Chen et al. 2001). Conversely, downregulation of BDNF is associated with stress exposure and depression (Karege et al. 2002). Immobilization stress results in decreased BDNF in the hippocampus, an effect that is blocked by antidepressant treatment (Nibuuya et al. 1995; Smith et al. 1995).

The relevance of BDNF to human depression is suggested by postmortem studies showing that BDNF levels as well as levels of TrkB are decreased in brains of suicide subjects (Dwivedi et al. 2005). There is also evidence that BDNF can regulate functions relevant to antidepressant-responsive behavior in rats. Chronic infusion of BDNF into posterior midbrain nuclei results in effects that are similar to those of antidepressants in forced swim and learned helplessness models of depression (Siuciak et al. 1997) and administration of BDNF directly into localized regions of the hippocampus similarly results in antidepressant-like effects in these behavioral models (Shirayama et al. 2002). Other studies, however, have demonstrated that BDNF in the mesolimbic dopamine system produces pro-depressive effects in behavioral models, demonstrating region-specific actions of this neurotrophic factor (Berton et al. 2006; Eisch et al. 2005).

These findings have suggested that it might be possible to identify components of BDNF-regulated signaling pathways that underlie behavioral effects in rodent models of depression. BDNF exerts its predominant effects via activation of the high-affinity trkB tyrosine kinase receptor, which couples to a number of signaling pathways. One of the best-studied BDNF-regulated signaling cascades is activation of the MAP kinase or extracellular signal-regulated kinase (ERK) pathway (Huang and Reichardt 2003). This cascade is mediated by Ras-mediated activation of a MAPK kinase (MEK) that phosphorylates and activates ERK. Two reports have implicated components of ERK/MAP kinase signaling in antidepressant behavioral effects. Transgenic mice overexpressing a dominant negative isoform of trkB (trkB.T1 mice) were shown to be insensitive to the behavioral effects of imipramine and fluoxetine in a forced swim test (FST; Saarelainen et al. 2003), indicating a requirement for functional trkB receptors for antidepressant responsiveness in this test. In addi-
tion, previous studies from our group have demonstrated a requirement for MAP kinase signaling through MEK in the antidepressant effectiveness of intrahippocampally administered BDNF in depression models (Shirayama et al. 2002).

In this study, we tested the hypothesis that it might be possible to modulate behavior in antidepressant-responsive behavioral paradigms by directly modifying the function of the MAPK pathway. We used a pharmacologic inhibitor of MEK to investigate a potential role for MAP kinase signaling in mouse models of depression and assessed a role for this pathway in mediating behavioral responsiveness to antidepressant drugs.

Methods and Materials

Animals

Male C57Bl/6, DBA2/J, and BDNF heterozygous knockout mice were obtained from Jackson Laboratories (Bar Harbor, Maine) and were group housed in standard mouse cages with ad libitum access to food and water. Mice were obtained at 8–10 weeks of age and allowed a minimum of 2 weeks to acclimate before use in experiments. Mice were maintained on a 12-hour light–dark schedule with lights on at 7 AM. For all experiments, mice were randomly assigned to treatment conditions before the start of the experiment and were tested in counterbalanced order. Animal maintenance and use procedures were in accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by the Yale University Animal Care and Use Committee.

Drugs

Desipramine hydrochloride (Sigma, St. Louis, Missouri), citalopram hydrochloride, fluoxetine hydrochloride (gifts from Eli Lilly, Indianapolis, Indiana), and sertraline hydrochloride (gift from Pfizer, Groton, Connecticut) were prepared immediately before use and injected intraperitoneally (IP) or subcutaneously (SC) in a volume of 10 mL/kg. Doses were calculated as milligrams per kilogram of the salt form. PD184161 (gift from Pfizer) and SL 327 (gift from J.M. Trzaskos, Dupont Research Labs, Wilmington, Delaware) were dissolved in DMSO. Control animals received injections of .9% saline, DMSO, or both.

Behavioral Tests

Forced Swim. The FST was performed according to standard published procedures with minor modifications (Caldarone et al. 2003). Mice were placed in a glass cylinder (12 cm diameter) filled to a depth of 10 cm with water (23–25°C). A 6-min swim test session was videotaped, and the time spent immobile during the last 4 min was recorded by an observer blinded to treatment or genotype groups. Immobility was defined as lack of all movement except for whisker movement and respiration. The immersion test session was videotaped, and the time spent immobile during the last 4 min was recorded by an observer blinded to treatment or genotype groups. Immobility was defined as lack of all movement except for whisker movement and respiration. The time of the start of the 6-min swim test was determined by the tip of the tail and is suspended 50 cm from the benchtop within a visually isolated area. Immobility time (defined as lack of all movement except for whisker movement and respiration) is recorded throughout a 6-min test session.

Locomotor Activity. Locomotor activity was measured by video tracking software (Ethovision software, Noldus, Alexandria, Virginia) in standard mouse cages.

Immunoblotting. Dissected hippocampus was immediately frozen on dry ice then sonicated in 1% sodium dodecyl sulfate. Total protein (20 mg) was added in each lane for immunoblot analysis with a phospho-ERK antibody (Cell Signalling Technology) using standard procedures (Kodama et al. 2005). Uniformity of loading was confirmed by reprobing with an antibody against total ERK (Santa Cruz Biotech).

Statistics

For analysis of two groups, data were subjected to Student’s t test. For analysis of effects of antidepressants and PD184161, data were analyzed by one-way analysis of variance (ANOVA) or two-factor ANOVA. Fischer’s protected least significant difference was used for post hoc pair wise comparisons of group means. Locomotor activity data were analyzed by ANOVA with repeated measures. In all cases comparisons were considered statistically significant for p < .05.

Results

Blockade of MEK Blocks the Phosphorylation of ERK

To confirm that systemic administration of the MEK inhibitor PD184161 results in inhibition of ERK phosphorylation, we injected mice with 10, 25, or 50 mg/kg PD184161 (IP), and 1 hour later, hippocampal tissue was collected for determination of phospho-ERK levels. As seen in Figure 1, PD184161 dose-dependently decreased basal levels of phospho-ERK 42/44 in the hippocampus without altering the amount of total ERK. We also

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not appear to measure equivalent processes (see Cryan et al 2003). These tests all measure stress-based responses but do not measure anticipation of helplessness or depressive-like activity and that are sensitive to acute drug treatments. We used three behavioral paradigms that are widely used to detect antidepressant-like activity in mouse models for depression, the learned helplessness (LH) paradigm quantifies the magnitude of an escape deficit resulting from prior exposure to inescapable shock (IES). Escape deficits are measured as failure to escape and latency for successful escapes, and depend on the prior shock exposure being inescapable (Maier and Seligman 1976). Escape deficits induced by IES are sensitive to reversal by antidepressant treatments (Shanks and Anisman 1989). Deficits do not occur if an equivalent amount of shock exposure is given in a way that can be controlled by the animal. Mice that are habituated to a training chamber with no footshock typically escape repeatedly with short latency when tested for response to footshocks on a subsequent test day. We assessed the influence of administration of PD184161 on subsequent LH escape performance of habituated and IES-exposed mice. The level of escape responding in habituated animals was not altered by acute PD184161 administration (30 mg/kg, IP; Figure 2). To be able to measure either increased or decreased LH escape performance by PD184161 in footshock-exposed mice, we used a submaximal level of IES exposure for the IES-exposed groups. This IES training did not result in significantly elevated escape latencies in the subsequent LH escape test in vehicle-injected mice. This submaximal level of IES exposure resulted in significantly increased escape deficits in the group of mice that also received acute PD184161 (1 hour pretest, Figure 2). Thus, the combination of the submaximal IES exposure plus a subthreshold dose of PD184161 (without effect in habituated mice) resulted in increased helplessness or depressive-like responses in LH testing. This effect of PD184161 administration is opposite to the effect of antidepressant administration on LH testing (Caldarone et al 2003).

We also tested the effect of acute administration of PD184161 on immobility in the FST. PD184161 administered 1 hour before the swim test significantly increased immobility. Similar to the results in learned helplessness, this depressive-like effect of the MEK inhibitor was opposite in direction to the effects of antidepressant drugs in this test (Figure 2). We also tested the effect of the MEK inhibitor on performance in the TST. The immobility of PD184161-treated animals was not significantly different from that of vehicle-treated mice when immobility was quantified over the entire 6-min test period. Although a 6-min TST is useful for detecting antidepressant activity, pro-depressive effects are more difficult to detect in the latter portion of this test when levels of immobility increase in control animals. A pro-depressive effect of acute PD184161 administration was evident, however, during the initial 3 min of the test and was significantly different from the level of immobility of vehicle-treated mice (Figure 2). Immobility behavior is submaximal and still developing in control animals during the first portion of the TST suggesting the relevance of the early part of this test as appropriate for assessing pro-depressive effects.

MEK Inhibition Produces Depressive-Like Effects in Three Mouse Models of Depression

We were interested in how inhibition of MEK signaling might alter antidepressant responses in mouse models for depression, so we first investigated the influence of administration of MEK inhibitor alone on the behavioral responses of C57Bl/6 mice. We used three behavioral paradigms that are widely used to detect antidepressant-like activity and that are sensitive to acute drug treatments. These tests all measure stress-based responses but do not appear to measure equivalent processes (see Cryan et al 2003; Duman and Monteggia 2006). The learned helplessness (LH) paradigm quantifies the magnitude of an escape deficit resulting from prior exposure to inescapable shock (IES). Escape deficits are measured as failure to escape and latency for successful escapes, and depend on the prior shock exposure being inescapable (Maier and Seligman 1976). Escape deficits induced by IES are sensitive to reversal by antidepressant treatments (Shanks and Anisman 1989). Deficits do not occur if an equivalent amount of shock exposure is given in a way that can be controlled by the animal. Mice that are habituated to a training chamber with no footshock typically escape repeatedly with short latency when tested for response to footshocks on a subsequent test day. We assessed the effect of administration of PD184161 on subsequent LH escape performance of habituated and IES-exposed mice. The level of escape responding in habituated animals was not altered by acute PD184161 administration (30 mg/kg, IP; Figure 2). To be able to measure either increased or decreased LH escape performance by PD184161 in footshock-exposed mice, we used a submaximal level of IES exposure for the IES-exposed groups. This IES training did not result in significantly elevated escape latencies in the subsequent

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Figure 1. PD184161 dose-dependently inhibits phosphorylation of extra-cellular signal-regulated kinase (ERK). Bar graph shows a dose-dependent decrease in ERK phosphorylation as determined by optical density analysis of Western blots from hippocampal tissue. PD184161 was administered at the doses indicated by intraperitoneal injection 1 hour before sacrifice. Each bar represents the mean ± SEM of four separate determinations. Representative images of phospho-ERK and total ERK are shown in the lower panels. *p < .02 versus vehicle.

Figure 2. PD184161 produces depressive-like effects in three behavioral models. In the learned helplessness paradigm, an intermediate level of inescapable shock training (subthreshold for inducing helplessness) results in escape latencies in vehicle-treated mice that are not significantly different from those of habituated mice (no shock exposure). Mice that were exposed to the same level of shock training and received a single injection of PD184161 (30 mg/kg intraperitoneal [IP], 1 hour before escape testing) had escape latencies that were significantly increased compared with latencies of similarly trained vehicle-treated mice. *p < .01 for PD184161 versus vehicle treated; n = 10–12 per group. Bars represent means ± SEM, pooled from two separate experiments. In the forced swim test, acute administration of PD184161 (30 mg/kg, IP, 1 hour before swim test) resulted in significantly increased immobility compared with vehicle treatment. *p < .02; n = 10 per group pooled from two separate experiments. In the tail suspension test, acute administration of PD184161 (30 mg/kg, IP, 1 hour before test) resulted in a significant increase in immobility compared to vehicle treatment. *p = .02; n = six per group, pooled from two separate experiments. Tail suspension data were quantified for the first 3 min of the test.
was given 1 hour before the locomotor test and at a dose of 30 mg/kg to simulate the dose and time parameters used for the depression tests. The locomotor activity of PD184161-injected mice was increased relative to vehicle-injected mice at the initial time point tested (60 min postinjection) and remained significantly elevated for the additional time points tested up to 120 min (Figure 3). This dose of PD184161 also increased locomotor activity at 30 min postinjection, and similar elevation of locomotor activity was observed for doses of 10 and 50 mg/kg PD184161 (not shown). The acute locomotor activation resulting from PD184161 injection indicates that the increased immobility of PD184161-injected mice in depression tests is not a consequence of reduced activity levels. In fact, the results indicate that responding in the stress-related models of depression can be dissociated from the locomotor activity effects of this MEK inhibitor.

MEK Inhibition Blocks the Antidepressant Effects of Desipramine and Sertraline in the FST

Next we examined the influence of the MEK inhibitor on responses to antidepressant administration in the FST. Given that adaptive changes in signaling pathways are thought to be important to the efficacy of antidepressants, we administered PD184161 in a way that would allow for a more sustained effect on signaling and assessed the resulting influence on behavioral outcome.

We first assessed the influence of PD184161 administration on the antidepressant effect of desipramine (DMI) in the FST. DMI was administered in three doses over a 24-hour period similar to protocols used in previous studies (Conti et al. 2002). This treatment paradigm resulted in decreased immobility compared with vehicle treatment, illustrating an antidepressant effect (Figure 4a). PD184161 pretreatment significantly blocked this effect of DMI in the FST (Figure 4a). It is unlikely that the blockade of the DMI effect is simply due to additivity of the opposing actions of PD184161 for two reasons. First, PD184161 was coadminis-
immobility (data not shown). Second, this subacute administration of PD184161 alone resulted in a decrease in immobility indicating that the blockade of desipramine action by PD184161 is not due to an additive effect of PD184161. The decrease in immobility produced by PD184161 in this experiment is opposite to the actions of a single acute injection (Figure 2). The reason for different effects of acute and subacute treatment with PD184161 is unclear but could be the result of regional involvement that differs depending on the treatment paradigm.

In agreement with the literature (David et al 2003; Lucki et al 2001), we found that C57Bl/6 mice were not sensitive to the effects of serotonin-selective reuptake inhibitors (SSRIs) in the FST (shown for citalopram in Figure 4B). We therefore used DBA/2J mice (Lucki et al 2001) to assess modulation of SSRI effects by coadministration of PD184161. Significant antidepressant effects were produced by subacute administration of both DMI and the SSRI sertraline in DBA mice (Figure 4C and 4D).  Coadministration of PD184161 reversed the effects of DMI and partially reversed the effect of sertraline in this mouse strain (Figure 4C and 4D). PD184161 alone did not influence immobility in the DBA mice indicating that this blockade of the antidepressant response cannot be explained by a simple additive effect.

Inhibition MEK Blocks the Effects of Desipramine but Not SSRIs in the TST

The influence of MEK inhibition on antidepressant responses in the TST was also examined in both C57Bl/6 and DBA/2J mice. Desipramine produced a typical antidepressant-induced decrease in immobility and acute administration of PD184161 significantly diminished this effect in both strains (Figure 5A and 5D). For these experiments, we quantified the entire 6-min test period during which no significant effect of PD184161 alone was detected. This can be seen in Figure 5 (A and B) for C57Bl/6 mice, and similar results were obtained in a separate experiment for DBA/2J mice (data not shown). Although a significant depressive-like effect of PD184161 can be measured over the first 3 min of the TST (Figure 2), the increasing immobility of control mice as the 6-min test period progresses prevents a significant depressive-like effect of PD184161 from being detected over the

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**Figure 5.** PD184161 blocks antiimmobility effects of desipramine (DMI) but not fluoxetine (FLX) or citalopram (CIT) in the tail suspension test. (A) Administration of DMI decreases immobility in C7Bl/6 mice, and this effect is reduced by coadministration of PD184161. [ANOVA: significant effect of DMI, F(2,40) = 21.78, p < .0001. Significant effect of PD184161, F(1,40) = 12.82, p = .0009. No significant DMI × PD184161 interaction, F(2,40) = 6.9, p = .50. Fischer’s protected least significant difference (PLSD) post hoc test: *p < .0001 vs. vehicle, **p < .04 vs. corresponding DMI alone and not significantly different from vehicle, p > .05. †p < .02 vs. DMI alone and p = .04 vs. vehicle]. (B) Administration of fluoxetine decreased immobility in C57Bl/6 mice and this effect was not altered by coadministration of PD184161. [ANOVA: significant effect of FLX, F(3,49) = 22.93, p < .0001. No significant effect of PD184161, F(1,49) = .63, p = .43. No significant FLX × PD184161 interaction, F(3,49) = 3.3, p = .08. Fischer’s PLSD post hoc test: *The two bars at each dose of FLX are not different from each other and are significantly different (p = < .004) from vehicle]. (C) PD184161 blocks the effect of DMI but not the effect of CIT in the same experiment. [ANOVA: effect of group, F(4,16) = 7.81, p = .001. Fischer’s PLSD post hoc test: *p < .001 vs. vehicle, **p = .01 vs. DMI alone and p > .05 vs. vehicle]. (D) Administration of DMI decreases immobility in DBA/2J mice, and this effect is reduced by coadministration of PD184161. [ANOVA: effect of group, F(2,11) = 20.16, p = .0002. Fischer’s PLSD post hoc test: *p < .0001 vs. vehicle, †p = .02 vs. DMI alone and p = .008 vs. vehicle]. (E) Administration of sertraline decreased immobility in C57Bl/6 mice, and this effect was not altered by coadministration of PD184161. [ANOVA: effect of group, F(2,17) = 13.01, p = .0004. Fischer’s PLSD post hoc test: *p = .0002 vs. vehicle and not different from each other]. Immobility was quantified over the entire 6-min test period. Antidepressant drugs or saline were injected (intrapertoneally [IP]) at the doses indicated 30 min before the test. PD184161 (30 mg/kg, IP) was injected 60 min before the test (50 mg/kg PD184161 was used for the highest dose of FLX). Bars represent the mean ± SEM of 6–7 mice per group.

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entire test interval. Despite the low sensitivity of the 6-min quantification for detecting depressive-like effects, we were still able to show significant effects of PD184161 on the responses to desipramine.

The SSRIs citalopram and fluoxetine (tested in C57) and sertraline (tested in DBA) were effective in producing typical antidepressant-like, decreased immobility, but PD184161 did not significantly alter these effects (Figure 5B, C, and E). This lack of effectiveness of PD184161 in modifying SSRI effects was shown for citalopram in marked contrast to a robust blockade of the effect of DMI by PD184161 demonstrated within the same experiment (Figure 5C). Higher doses of PD184161 (40–50 mg/kg) were also ineffective in blocking the effects of these SSRIs (data not shown). Thus, in contrast to the FST in which PD184161 blocked the effects of both desipramine and an SSRI, the MEK inhibitor did not significantly alter SSRIs effects in the TST.

**MEK Inhibition Induces a Depressive Behavioral Phenotype in BDNF Mutant Mice**

Administration of BDNF can produce antidepressant-like behavioral effects in the rat FST (Shirayama et al 2002; Siuciak et al 1997). It is possible that a decrease in BDNF could result in a corresponding behavioral deficit in a swim test. Although BDNF heterozygous (BDNF +/-) mutant mice have half the amount of BDNF as wildtype (BDNF +/+ ) control mice, their performance in the FST indicates that alone this reduction is not sufficient to produce a behavioral deficit in vehicle-treated mice (Figure 6; Enfors et al 1994; Korte et al 1995; MacQueen et al 2001). It is possible that reduced levels of BDNF in the heterozygous mutant mice are not low enough to produce a depressive phenotype but that blockade of BDNF-ERK signaling could combine with the BDNF deficit to result in a measurable alteration in behavior. We used a pharmacogenetic approach described (Ohno et al 2001) to test this hypothesis.

This approach involves combining a recessive genetic mutation with a low-level pharmacologic manipulation to reveal the expression of a behavioral phenotype. We administered PD184161 to BDNF +/+ and +/- littermate control mice at a single subthreshold dose that does not elicit behavioral effects in wildtype mice. There was significant difference in the immobility of vehicle-injected BDNF +/+ compared with +/+ control mice; however, acute administration of a low dose of PD184161 influenced immobility in the BDNF +/- mice such that there was a significant increase in the immobility of PD184161-injected BDNF +/+ mice compared with PD184161-treated +/+ control mice (Figure 6a). We have replicated this result in BDNF +/- mice using another selective MEK inhibitor (SL327) also at a low dose that, alone, does not have significant behavioral effects (Atkins et al 1998; unpublished observation). Administration of low dose SL 327 (10 mg/kg, IP) also resulted in increased immobility in the BDNF +/- mice that was significant compared with SL 327-treated wildtype control mice (Figure 6b). Thus MEK inhibition can contribute to the appearance of a mild depressive-like phenotype in BDNF +/- mice.

This result indicates that disruption of the MEK signaling pathway reveals a depressive phenotype in mice that appear to be vulnerable because of a genetic deficiency in BDNF. We were interested in seeing whether exposure to an environmental challenge, chronic stress, revealed a similar depressive phenotype. We were able to achieve a low-level chronic stressor that, like the BDNF heterozygous deletion did not alone alter behavior in the FST. BDNF +/- and wildtype control mice were subjected to 3 weeks of isolation stress and then tested in the FST (Figure 6c). BDNF +/- mice that were subjected to the isolation stress showed an enhanced immobility in the FST that was not apparent in the wildtype control mice. These BDNF +/- and wildtype mice were found not to differ from each other in an initial swim test (mean immobility: 102 ± 10 BDNF +/-; 115 ± 12 wild-type) in agreement with previous observations from this lab and published reports (MacQueen et al 2001). We interpret the enhanced immobility of BDNF +/- mice to be due to a greater behavioral vulnerability of the BDNF +/- mice to the combination of swim and isolation stress. This suggests that BDNF-related mechanisms could be involved in the actions of stress and result in depressive behavioral changes in the FST. Alternatively, it is possible that stress exposure could act through mechanisms independent of BDNF-MAP kinase signaling that are functionally additive with a BDNF deficit.

**Discussion**

To test the hypothesis that intracellular signaling through MEK contributes to behavioral actions of antidepressant drugs, we investigated the influence of MEK inhibition. The results demonstrate that pharmacologic inhibition of MEK acutely results in depressive-like effects in behavioral models of depression. In addition, the behavioral effects of antidepressants were blocked by concurrent administration of a MEK inhibitor, but SSRIs differed from desipramine in the dependence of their behavioral effects on MEK signaling. Also, our results demonstrate that mice with a heterozygous deletion of the BDNF gene show a depressive phenotype when challenged with a low dose of MEK inhibitor or with a stressor, although neither the gene deletion nor the low-level challenges alone result in observable behavioral changes. These results support a role for intracellular signaling through MEK-ERK in the regulation of behavior in mouse models of depression, including the regulation of this behavior by BDNF and by stress.

Pharmacologic inhibitors of MEK have been useful in identifying roles for MAPK signaling in synaptic plasticity and memory consolidation in a variety of systems (Atkins et al 1998; Gerdjikov et al 2004; Kelly et al 2003; Ribeiro et al 2005; Sharma et al 2003; Schweitzer et al 1998; Pereira et al 2002). For our studies, we have used the novel compound PD184161, which is reported to be a specific and more potent MEK1 inhibitor (Davies et al 2000; Yung et al 2004). We found that PD184161 administration produced a dose-dependent inhibition of ERK phosphorylation in hippocampus. A dose of 30 mg/kg that is submaximal for inhibition of ERK phosphorylation was chosen for behavioral studies to reduce the potential for nonspecific effects.

Acute administration of PD184161 produced depressive-like responses in learned helplessness, FST, and the TST, effects that are opposite to the actions of antidepressant drugs. This indicates that the MAP kinase pathway is necessary for normal behavioral responding in these three tests. Because depressive-like effects in these tests consist of reduced movement or immobility, it is possible that a drug-induced decrease in mobility could contribute to the observed response. We found, however, that acute administration of PD184161 actually increased locomotor activity, indicating that the behavioral effects that we observed after acute MEK inhibition cannot be interpreted as an artifact of
Figure 6. MEK inhibition and stress increase the immobility of brain-derived neurotrophic factor (BDNF) mutant mice in the FST. (A) The immobility of BDNF (+/−) mice injected with vehicle was not different from the immobility of vehicle-treated control (+/+ ) mice in the day 2 swim test. The immobility of BDNF (+/−) mice injected with PD184161 (10 mg/kg, intraperitoneal [IP] injection) was increased compared with the immobility of PD184161-injected control (+/+) mice. [ANOVA: Significant effect of genotype, \( F(1,45) = 8.54, p = .005 \). No significant effect of PD184161, \( F(1,45) = 2.27, p = .14 \). No significant genotype × PD184161 interaction \( F(1,45) = .174, p = .68 \). Fischer’s protected least significant difference (PLSD) post hoc test: * \( p = .02 \) for PD184161-injected BDNF (+/−) vs. BDNF (+/+). Each bar is the mean ± SEM, \( n = 11–14 \) per group pooled from two separate experiments. PD184161 or vehicle were injected 1 hour before the day 1 swim. (B) Increased immobility of BDNF (+/−) mice in comparison with control (+/+) mice was also seen after injection with a low dose (10 mg/kg) of another MEK inhibitor, SL 327, in the same paradigm. [ANOVA: Significant effect of genotype, \( F(1,45) = 4.97, p = .04 \). No significant effect of SL 327 \( F(1,24) = .16, p = .69 \). No significant genotype × SL 327 interaction \( F(1,24) = .79, p = .38 \). Fischer’s post hoc test: * \( p = .02 \) for SL 327-injected BDNF (+/−) vs. BDNF (+/+). Results are presented as the mean ± SEM, \( n = 5–9 \) per group pooled from two separate experiments. (C) BDNF (+/−) mice show increased immobility in the forced swim test compared with wildtype littermate control mice (+/+) after 21 days of isolation housing. [Student’s t test: * \( p = .0014 \) compared with control (+/+)]. Results are the mean ± SEM, \( n = 11 \) per group, pooled from two separate experiments.

drug-induced locomotor activity. Another study showed decreased immobility resulting from acute MEK inhibition in the rat FST; however, drug-induced locomotor activation could have contributed to that result (Eimat et al 2003).

To test the role of MAPK signaling in the actions of antidepressants in the FST, we chose a subacute treatment paradigm that includes a swim stress session on day 1 followed by 3 antidepressant treatments and 2 PD184161 doses before testing on day 2. Our reasoning was that the MAPK pathway could be involved in the adaptive changes resulting from exposure to stress, repeated antidepressant treatment, or both. We found that the behavioral effects of desipramine and sertraline in the FST were significantly reduced by coadministration of PD184161, although the inhibitor did not completely reverse the effects of the SSRI. The results indicate that the actions of these two major classes of antidepressants, 5-HT and norepinephrine (NE) selective reuptake inhibitors, are mediated, at least in part by activation of the MAPK signaling pathway. This is consistent with the results of Saarelainen et al (2003) that showed a requirement for BDNF-TrkB signaling in the effects of antidepressants in the FST using a dominant negative TrkB transgenic mouse line. In these FST experiments, subacute (injections on two consecutive days) PD184161 administration resulted in an antidepressant-like effect in C57Bl/6 mice. The reasons for the difference in acute versus subacute effects of PD184161 in the FST are unclear. One possibility is that behavior can be influenced by multiple brain regions and that the contribution of these regions is differentially influenced by PD184161 depending on the length of treatment. For example, BDNF infusions into the ventral tegmental area of the mesolimbic dopamine system increase immobility in the FST (Eisch et al 2003), and BDNF is required for development of experience dependent social aversion, an effect that is reversed by antidepressant treatment (Berton et al 2006). It is possible that the antidepressant-like effects observed with subacute PD184161 in our study result from differential blockade of ventral tegmental area BDNF signaling; however, our results indicate that the prevailing actions of the MEK-ERK signaling cascade are to support an antidepressant like behavioral response. The depressive actions may only be observed under limited conditions.

The mediation of behavioral effects by brain regions in addition to hippocampus could also explain the presence of an antidepressant behavioral response seen in one report in CREB deletion mutant mice. That study showed a behavioral effect of desipramine in the mouse FST in a CREB deletion mutant mouse that does not show desipramine-regulation of BDNF in hippocampus (Conti et al 2002). In addition to the possibility of brain areas in addition to hippocampus mediating such effects, the likelihood of compensatory responses that counteract the actions of gene deletion, cannot be excluded with constitutive null mutant mice like that CREB deletion line.

Our studies in the TST suggest a differential involvement of the MAPK pathway in the actions of NE versus 5-HT selective reuptake inhibitors. We found that administration of the MEK inhibitor significantly blocked the effect of desipramine, examined in both C57 and DBA mice. In contrast, the MEK inhibitor did not significantly alter the antidepressant actions of three SSRIs, tested in the two strains of mice. These data indicate a difference in the mechanisms underlying the actions of NE and 5-HT reuptake inhibitors in the TST versus the FST. This difference is also highlighted by the partial blockade of desipramine in the TST, compared with the complete blockade of the NE selective reuptake inhibitor by PD184161 in the FST. Other studies have also found that the TST and FST do not always yield the same results, suggesting that the processes responsible for generating the behavioral output in these two tests are not identical (Bai et al 2001; Bourin et al 2005; Holmes et al 2002). Other evidence that NE and 5-HT selective reuptake inhibitors differ in their interactions with MEK signaling is reported in a study by Fumagalli et al (2005) showing ERK activation induced by fluoxetine treatment differs from that resulting from imipramine, consistent with our behavioral data. It is also possible that the differences observed are due to the subchronic PD184161 treatment paradigm used for the FST versus the single acute dose.
used for the TST. The acute single dose may not be sufficient to block
the effects of the SSRI or to block completely the NE
selective antidepressant in the TST. Future studies are being
designed to test this possibility by developing a TST paradigm
that includes a prestress component.

Our results implicate involvement of this BDNF-activated
signaling pathway in the behavioral effects of antidepressants but
do not directly address a role for BDNF in behavior in these tests.
We have previously reported that central administration of a MEK
inhibitor (U0126) blocks the antidepressant effect of intrahippocampally administered BDNF in the FST (Shirayama et al
2002). The behavioral effects of antidepressants in the FST are
blocked in mice deficient in BDNF and in TrkB dominant negative transgenic mice (Monteggia et al 2004; Saarelainen et al
2003). Taken together the results suggest that a BDNF-activated
TrkB signaling pathway is activated by antidepressant drugs.

The results of this study demonstrate that blockade of MAPK
signaling blocks the actions of antidepressant treatment; how-
ever, the question of the extent to which antidepressant drugs induce measurable changes in components of ERK/MAPK sig-
naling remains unresolved. Literature reports of changes in ERK
activation after acute antidepressant treatment have been vari-
able and inconclusive. It is possible that actions on ERK signaling
differ for different classes of antidepressants as suggested from
reports on chronic drug treatment (Fumagalli et al 2005; Tirabos-
chi et al 2004) and studies are presently underway in our lab to
test this hypothesis. The report that antidepressant treatment
increases the phosphorylation of TrkB at a site not associated with
activation of the MAPK pathway also further demonstrates a
role for BDNF-TrkB signaling but suggests that the nature of the
MAPK activation requires further elucidation (Saarelainen et al
2003).

Our findings are consistent with the hypothesis that MAPK
signaling contributes to the actions of antidepressants and BDNF.
To further examine the interplay between BDNF and MAPK signal-
ing, we tested behavioral responses in BDNF mutant mice. Mutant
mice heterozygous for a BDNF deletion (BDNF +/-) have BDNF
concentrations that are half that of wildtype mice, and unlike the
homozygous knockouts, heterozygous mice have a normal life
span and no obvious sensorimotor deficits (Conover et al 1995;
Enfors et al 1994; Korte et al 1995). BDNF +/- mice show a pro-
dressive deterioration of forebrain 5-HT neurons and show
behavioral alterations, including increased aggression that is alle-
viated by chronic SSRI treatment, as well as hyperphagia and pro-
gressive obesity (Lyons et al 1999). BDNF +/- mice have not been
defined as a depressive phenotype (Chourbaji et al 2004; MacQueen et al 2001; Saarelainen et al 2003). Conditional
BDNF null mutant mice with selective, postnatal deletion of
BDNF in forebrain areas also show increased aggression and
hyperphagia (Rios et al 2001), altered or no change in models of
anxiety (Rios et al 2001; Gorski et al 2003) and no change in
baseline FST performance (Monteggia et al 2004).

In our study, we did not observe a significant change in
baseline FST performance in BDNF +/- mice, consistent with
previous reports. Because alterations in BDNF levels are associ-
ated with changes in FST performance (Russo-Neustadt et al
2001; Shirayama et al 2002; Stucicak et al 1997), we speculated that
the reduction in BDNF in these mice is subthreshold for produc-
ing a behavioral change in the FST. If this is the case, we further
reasoned that blockade of BDNF signaling via the MAPK cascade
would reveal a significant behavioral phenotype. This pharma-
cogenetic strategy has been described previously and used for
studies of learning and memory (Olino et al 2001). We found that
administration of a low dose of MEK inhibitor, which alone does not
produce changes in FST performance, produced a depressive-like
phenotype in BDNF +/- mice compared with BDNF +/- mice in
the FST. This result demonstrates a genetic vulnerability in the
BDNF +/- mice that is revealed by a pharmacologic challenge and
identifies a potentially useful animal model for studying depressive behavior in mice that are not significantly impaired
developmentally.

We also examined the potential vulnerability of the BDNF
heterozygous mice using another type of environmental chal-
lenge, chronic isolation stress. Mice exposed to chronic social
isolation undergo physiologic changes and show a greater propensity for psychopathology in rodent behavioral models for
psychiatric disorders (Cabbil et al 2002; Chida et al 2005; Guidotti
et al 2001; Spani et al 2003). Stress exposure is a known
precipitant of depression in humans (Caspi et al 2003; Kendler et
al 1999; Kessler 1997) and in animal models (Anisman and Matheson 2005; Henn and Vollmayr 2005) and can lead to a
reduction in the expression of BDNF in limbic brain regions
(Smith et al 1995). When BDNF heterozygous mice were ex-
posed to chronic isolation stress a significant depressive pheno-
type was revealed, demonstrating a synergistic interaction be-
tween stress and the BDNF deficit. In contrast, no such effect was
observed in the wildtype littermates. These results illustrate an
example of how a defined genetic alteration can confer
vulnerability to an environmental challenge and result in a
depressive behavioral phenotype. This type of gene x stress
interaction has been reported for a 5-HT transporter polymor-
phism in which individuals with the vulnerable allele are only
affected if they are also exposed to stressful life events (Caspi
et al 2003; Levinson 2005). A similar gene x stress interaction has
recently been demonstrated for BDNF in which children carrying
the BDNF Val66Met polymorphism and who are maltreated have
a higher incidence of depression (Kaufman et al 2006).

It is tempting to speculate that the stress exposure interacts
with the gene deletion possibly at the level of the signaling
pathway for BDNF. Studies in the literature have reported various
effects of stress exposure on ERK activation. Acute restraint stress
has been shown to increase ERK phosphorylation in hippocam-
pus and cortical areas (Meller et al 2003; Sasaguri et al 2005), and
acute swim was shown to increase ERK phosphorylation in
cortex and striatum but not in hippocampus or amygdala (Shen
et al 2004). Chronic footshock stress was reported to increase
ERK phosphorylation in dendrites of PFC neurons (Trentani et al
2002), although in another study, chronic restraint stress de-
creased ERK phosphorylation in PFC (Meller et al 2003). The
nature of the interaction of stress with ERK signaling is clearly
complex and varies depending on the stress exposure and the
brain regions examined. Definitive studies on the effects of stress
on ERK activation are warranted and are currently underway in
our laboratory.

In summary, the results of this study demonstrate that acute
blockade of MEK-ERK signaling produces a depressive-like
phenotype in three models of depression and blocks or reduces
the effects of both norepinephrine and 5-HT selective reuptake
inhibitors in the FST. Inhibition of MEK-ERK signaling also
reveals a genetic vulnerability for a depressive-like phenotype in
BDNF heterozygous mice, a vulnerability that is also expressed
upon exposure to stress. These findings suggest that MEK-ERK
signaling contributes to the behavioral actions of BDNF, and
possibly other neurotrophic factors that activate this pathway,
and to the actions of antidepressants. These results indicate that
drugs that activate the MEK-ERK pathway should produce an
antidepressant response, and studies are currently underway to
test this hypothesis (i.e., inhibition of the protein phosphatases
that inactive MEK and ERK). In addition, the results raise the
possibility that genetic variations in the BDNF gene, or in the
MEK or ERK genes, could contribute to genetic vulnerability that
results in depression upon exposure to stressful life events.
Studies to examine interactions of BDNF, MEK, or ERK gene
polymorphisms with stressful life events will be necessary to test
this hypothesis (Dwiведi et al 2001).

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