for by soil dust and the other half by Pb mining. Sample 2p2 has a $^{206}Pb/^{207}Pb$ = 1.1827 and its age lies between 3000 (145 cm, core 2p) and 2110 ^{14}C yr BP (96 to 102 cm, core 2f). If we assume that the background soil dust signature at this time was $^{206}Pb/^{207}Pb$ = 1.1999 (Fig. 3), we can calculate the isotopic composition of the Pb ores by solving for x: 1.1827 = 0.5 (1.1999) + 0.5 (x). Using this simple approach, we calculated that the Pb ores must have had $^{206}Pb/^{207}Pb$ = 1.1655. According to J. O. Nriagu (*2*), mining in the Iberian Peninsula accounted for 37% of the Pb that was produced during the Iron Age (1200 to 50 B.C.), making it the most important Pb mining area of its time. Ores from these mines are known to have $^{206}Pb/^{207}Pb$ values between 1.1722 and of 1.1619 (*58*). The single most important ore body from this area is Rio Tinto, and the galenas from this ore range from $^{206}Pb/^{207}Pb$ = 1.1632 to 1.1639 [C. Pomiès, A. Cocherie, C. Guerrot, E. Marcoux, J. Lancelot, *Chem. Geol.* **144**, 137 (1998)].

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Distinct Mechanism for Antidepressant Activity by Blockade of Central Substance P Receptors

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The localization of substance P in brain regions that coordinate stress responses and receive convergent monoaminergic innervation suggested that substance P antagonists might have psychotherapeutic properties. Like clinically used antidepressant and anxiolytic drugs, substance P antagonists suppressed isolation-induced vocalizations in guinea pigs. In a placebo-controlled trial in patients with moderate to severe major depression, robust antidepressant effects of the substance P antagonist MK-869 were consistently observed. In preclinical studies, substance P antagonists did not interact with monoamine systems in the manner seen with established antidepressant drugs. These findings suggest that substance P may play an important role in psychiatric disorders.

The development of new drugs to treat depression has been severely constrained by a poor understanding of the pathophysiology of this disease and of the mechanisms by which drugs that augment monoamine function alleviate its symptoms. The predictive validity of many preclinical assays is also limited by an inability to model psychiatric disease in animals. However, there is a pressing need for improved antidepressant therapies, given the considerable prevalence, morbidity, and mortality of depressive disorders, the incomplete efficacy of currently available drugs in many patients, and the potentially distressing adverse effects of existing therapies (*1*).

Localization of substance P in brain: Evidence for an involvement in the response to stress. Substance P is the most abundant neurokinin in the mammalian central nervous system (CNS). Mapping studies indicate that the substance P–preferring neurokinin-1 (NK_1) receptor is highly expressed

in brain regions that are critical for the regulation of affective behavior and neurochemical responses to stress (*2*). This distribution provides multiple opportunities for interactions between substance P and the convergent norepinephrine and serotonin pathways through which established antidepressant drugs act, suggesting that substance P antagonists might have utility in the treatment of psychiatric disorders. Some norepinephrine- and serotonin-containing cell bodies also coexpress substance P, presenting opportunities for more direct neuronal modulation (*2*). The potential for such functional interactions in vivo is supported by the observation that repeated administration of established antidepressant drugs causes down-regulation of substance P biosynthesis in discrete brain regions in rats, raising speculation that alterations in neurokinin systems may contribute to their antidepressant efficacy (*3*).

Activation of central substance P pathways occurs in response to noxious or aversive stimulation. Neurochemical experiments in rats revealed changes in substance P content in the hippocampus, septum, periaqueductal gray, and ventral tegmental area after inescapable foot shock, immobilization, and social isolation (*4*). Central injection of substance P or related peptide agonists induces conditioned place aversion and produces an anxiogenic profile on the elevated plus maze, implying that activation of central substance P pathways is aversive (*5*). There is, however, little direct or experimental evidence that overactivity in central substance P pathways may be involved in the pathophysiology of depression or anxiety. In one study, higher concentrations of substance P were found in the cerebrospinal fluid of depressed patients (*6*); however, this finding was not replicated (*7*).

Development and characterization of nonpeptide substance P antagonists. Since the discovery of the first nonpeptide substance P receptor antagonist, CP-96,345 (*8*), several groups have produced structurally diverse, highly selective antagonists. This created the opportunity to investigate whether selective blockade of central substance P receptors is capable of modifying responses to stress in preclinical studies (*9*). Recently, we described the synthesis of the bis(trifluoromethyl) morpholine MK-869, an orally bioavailable, long-acting substance P antagonist (*10*) that was selected for clinical development. An analog of this compound, L-760,735 (*11*), and the structurally unrelated agent L-733,060 (*12*) were used as research tools in our preclinical studies because of the availability of chemically related compounds with low affinity for the substance P receptor (L-770,765 and L-733,061, respectively) that could be used to control for nonspecific pharmacological effects. By comparing the profiles of these compounds, we were able to ensure that the effects we observed in preclinical assays were really attributable to blockade of the substance P receptor.

MK-869 and L-760,735 exhibited high affinity for the gerbil and guinea pig NK_1 receptor (mean inhibitory concentration IC_{50} = 0.3 to 0.5 nM) (*13*), and the preclinical profile of these compounds was therefore characterized using these species. The selectivity of MK-869 and L-760,735 for the human substance P receptor was much greater than for 90 other G protein–coupled receptors and ion channels (by a factor of ≥3000); no significant activities of the parent compounds or their metabolites were detected against monoamine oxidase A or B, norepinephrine, dopamine, and serotonin reuptake sites, 5-hydroxytryptamine ($5\text{-}HT_{1A}$ or $5\text{-}HT_{2A}$) receptors, monoamine transporters, or mu, delta, or kappa opiate receptors ($IC_{50} \geq 3$ μM) (*13*).

In gerbils, central infusion of substance P agonists, such as GR73632, elicits a vigorous and readily quantifiable rhythmic drumming or tapping of the hind feet, which can be inhibited by systemic administration of brain-penetrant substance P receptor antagonists (*14*). Thus, inhibition of NK_1 agonist–induced foot tapping in this species provides an in vivo functional assay for the CNS penetration of antagonists, and this enabled us to identify optimal research tools with which to investigate the role of substance P in the brain. MK-869, L-760,735, and L-733,060 all potently inhibited GR73632-induced foot tapping (mean inhibitory dose $ID_{50} \leq 0.3$ mg/kg of body weight, intravenously).

Antidepressant-like profile of substance P antagonists in preclinical assays. In guinea pigs, central infusion of substance P agonists causes locomotor activation (*15*) accompanied by pronounced and long-lasting audible vocalizations (*16*). This observation was of particular interest because psychotropic drugs that alleviate symptoms of anxiety and depression in humans are known to inhibit stress-induced vocalizations in many mammalian species (*17*). In guinea pigs, vocalizations elicited by intracerebroventricular (icv) infusion of GR73632 (0.1 nmol) were virtually abolished by pretreatment with L-733,060 (3 mg/kg), but not by its less active enantiomer L-733,061, confirming the NK_1 receptor specificity of this response. GR73632-induced vocalizations were markedly attenuated (>70%) by acute pretreatment with the antidepressant drugs imipramine and fluoxetine (30 mg/kg), but not by the anxiolytics diazepam (3 mg/kg) or buspirone (10 mg/kg) (Fig. 1). These findings show that clinically used antidepressant drugs were able to block the behavioral effects of central substance P receptor stimulation.

We then investigated the involvement of endogenous substance P release in vocalization caused by psychological stress in this species. The ability of the substance P antagonists L-760,735, L-733,060, and their low-affinity analogs to inhibit vocalizations evoked in guinea pig pups by transient maternal separation was compared with that of clinically used antidepressant and anxiolytic drugs. During 15 min of separation from their mothers and littermates, guinea pig pups emitted an audible vocalization response resembling that elicited by central infusion of GR73632. Consistent with findings in this and other species (*17*), acute administration of the antidepressant drugs phenelzine, imipramine, or fluoxetine, or of the anxiolytics diazepam (a benzodiazepine) or buspirone (a

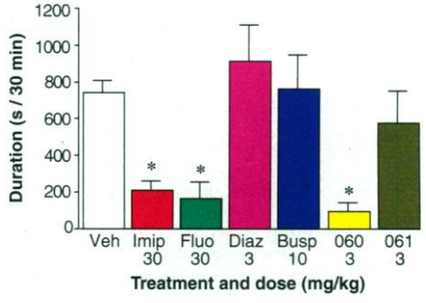

Fig. 1. Inhibition of vocalization induced by infusion of the substance P agonist GR73632 (0.1 nmol icv) in guinea pigs (*16*). Compounds were administered subcutaneously or intraperitoneally 30 min before the infusion. Imipramine (Imip), buspirone (Busp), L-733,060 (060), and L-733,061 (061) were dissolved in 0.9% saline and administered subcutaneously. Fluoxetine (Fluo) and diazepam (Diaz) were suspended in 0.5% methocel and administered intraperitoneally. Data were subjected to ANOVA followed by Dunnett's *t* test (n = 4 to 6 per group); *$p \leq 0.05$ compared with vehicle treatment.

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5-HT_{1A} receptor partial agonist), 30 min before maternal separation caused a dose-dependent and complete inhibition of separation-induced vocalizations in guinea pig pups. Similarly, the substance P antagonists L-760,735 and L-733,060 completely inhibited separation-induced vocalizations (Fig. 2). In contrast, compounds with weak NK_1 receptor affinity, L-770,765 (3 mg/kg) and L-733,061 (10 mg/kg), failed to inhibit separation-induced vocalizations (≤14%), again confirming the substance P receptor specificity of this effect. Inhibition of separation-induced vocalizations by substance P antagonists was critically dependent on their ability to penetrate the CNS because the poorly brain penetrant compounds L-743,310, LY 303870, and CGP 49823 (*14*) showed only weak activity in this assay (ID_{50} > 30 mg/kg intraperitoneally). The NK_1 receptor antagonists from our morpholine series also potently inhibited vocalizations in guinea pig pups when administered orally 4 hours before maternal separation (ID_{50} for MK-869 was 0.7 mg/kg, versus 0.9 mg/kg for L-760,735), indicating their suitability as oral therapeutic candidates. These studies demonstrate that selective pharmacological blockade of substance P receptors is capable of inhibiting behavioral responses to psychological stress in a manner resembling the effect of clinically used psychotherapeutic agents.

Demonstration that MK-869 is an efficacious and well-tolerated antidepressant in patients with major depressive disorder. A randomized double-blind placebo-controlled study was conducted to evaluate the safety and efficacy of single daily doses of 300 mg of MK-869 in comparison to paroxetine (20 mg) or placebo in outpatients with major depressive disorder (MDD) and moderately high anxiety. MK-869 was chosen to test the concept clinically because of its high affinity, selectivity, brain penetrance, duration, and oral bioavailability that permitted a once daily oral dosing regimen. MK-869 was well tolerated in human volunteer studies at 300 mg, a dose for which pharmacokinetic data predicted >90% blockade of central substance P receptors.

The study was conducted at four experienced investigative sites (*18*). Eligible patients completed a washout of previous psychotropic medications (*19*) and were randomized in equal numbers to receive MK-869, paroxetine, or placebo (*20*). Efficacy measurements were made at the end of weeks 1, 2, 4, and 6, or on termination. The primary efficacy outcome measure was the 21-item Hamilton depression (HAM-D21) total score; secondary measures included the Hamilton anxiety (HAM-A) total score and the Clinical Global Impressions severity scale (CGI-S) (*21*). The demographics of the patients in the three treatment groups were comparable (*22*).

The principal outcome was a 4.3-point difference in mean change from baseline to week 6 between MK-869 and placebo in total HAM-D21 score, confirming that MK-869 is an efficacious antidepressant (Fig. 3A). The effect of MK-869 was similar to that of paroxetine (mean change of 3.6 points). The antidepressant effect of MK-869 was observed at all four investigative sites [differences of 4.1, 6.6, 3.3, and 3.2 points, respectively (*22*)] and was corroborated by all secondary measures. A standard measure of response, ≥50% change from baseline to week 6 in total HAM-D21 score, was compared for the different treatment groups. Of the patients receiving MK-869, 54% showed improvements of ≥50% from baseline, compared with 46% for paroxetine and 28% in the placebo group. In addition, 43% of patients treated with MK-869 reached scores of <10 points (generally considered to be a complete response) on the HAM-D17 at last rating, compared with 33% of patients treated with paroxetine and 17% of patients receiving placebo.

Mean changes from baseline to week 6 for the four factors of the HAM-D21 showed that the antidepressant profiles of MK-869 and paroxetine were generally similar (*23*). Changes for each of the 21 items of the HAM-D21 were explored further to compare the profile of MK-869 with that of paroxetine. Both drugs were superior to placebo on many items of the HAM-D21. Patients receiving MK-869 showed more improvement than those on paroxetine on items of insomnia (early; item 4) and genital symptoms (item 14), whereas paroxetine showed more improvement than MK-869 on item 17, insight. MK-869 also demonstrated significant anxiolytic activity in this population of depressed patients. An anxiolytic effect was gradually observed, which continued to increase through week 6 (Fig. 3B).

The safety and tolerability of MK-869 were generally similar to placebo, except for mild and typically transient somnolence and asthenia, side effects also observed with paroxetine (Table 1). The most common clinical adverse experiences (AEs) observed in patients receiving MK-869 were headache (32%), somnolence (20%), nausea (18%), and asthenia/fatigue (14%); these were generally mild and transient. Nausea, which occurred in 29% of patients on paroxetine compared with 10% of those on placebo, was the chief AE causing discontinuation of treatment with paroxetine. Notably, the incidence of sexual dysfunction in patients receiving paroxetine (a problem observed with other serotonin reuptake inhibitors) was 26%, significantly greater than with MK-869 (3%) or placebo (4%). In addition, discontinuation as a result of clinical AEs was more frequent among patients receiving paroxetine (19%) than among patients receiving MK-869 (9%) or placebo (9%). There was no pattern in the types of clinical AEs that caused discontinuation in patients on MK-869 or placebo. There were no reports of drug-seeking behavior (symptoms of drug withdrawal or any other AEs suggestive of a potential for drug abuse) or clinically signif-

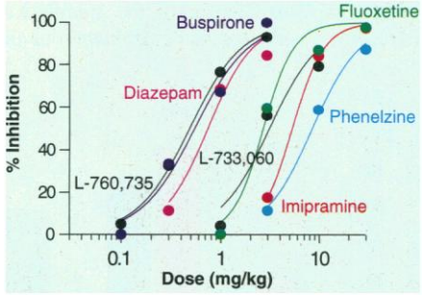

Fig. 2. Inhibition of vocalization induced by transient maternal separation of guinea pig pups. Pups were prescreened to ensure that a vocalization response was reproducibly elicited after maternal separation. Pups were placed individually in a room isolated from the home cage for 15 min, and the duration of vocalization was recorded. Animals vocalizing for ≥5 min were used for drug challenge studies. Each pup received a subcutaneous or intraperitoneal injection of test compound (as described for Fig. 1) and was returned to the home cage for 30 min before maternal separation, as described above. The duration of vocalization on the drug treatment day is expressed as a percentage of the pretreatment baseline value for each animal (n = 4 to 6).

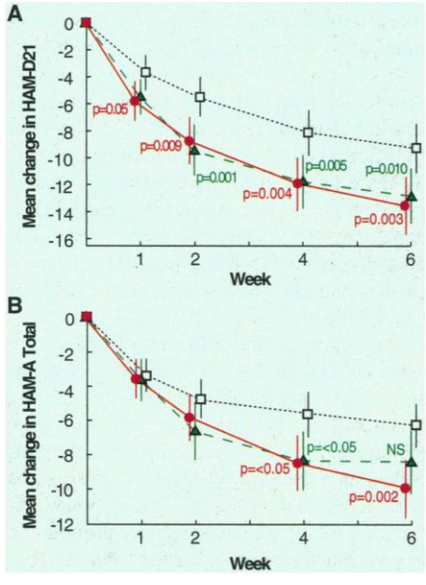

Fig. 3. Effect of treatment with MK-869 (300 mg/day) or paroxetine (20 mg/day) on mean change from baseline on the Hamilton Depression Scale (HAM-D21) (**A**) and the Hamilton Anxiety Scale (14 items) (**B**) in patients with major depressive disorder. Comparisons are of MK-869 (red circles, n = 66) or paroxetine (green triangles, n = 68) versus placebo (open squares, n = 64). Error bars show 95% confidence intervals.

icant changes in vital signs, physical examination, weight, or electrocardiograms in patients treated with MK-869 (*24*).

Novel mechanism of antidepressant activity. These findings provide clinical evidence that substance P antagonism represents a well-tolerated, distinct mechanism for antidepressant activity. The antidepressant effect of MK-869 was observed at all investigative sites and was consistently corroborated by all secondary measures—that is, HAM-D items (for example, item 1, depression), most HAM-D factors, percentages of patients achieving ≥50% response, percentages of patients with HAM-D scores of <10, and clinical global ratings. Because paroxetine showed a similar effect (mean change of 3.6 points) on the HAM-D21, the study had good assay sensitivity. In addition, the results of the "as observed" and "last observation carried forward" analyses were similar, indicating that the results were not markedly affected by carrying forward data in patients who discontinued (*25*). MK-869 also demonstrated significant anxiolytic activity in this population of depressed patients.

Anxiety levels of the depressed patients in this study were moderately high but within the range expected for a population with a primary diagnosis of depression. The differential time course of the antidepressant and anxiolytic effects (seen from visual inspection of the curves), and the similar profiles of MK-869 and paroxetine on the HAM-D21 factors and items, suggest that the antidepressant effects of MK-869 are independent of its potential anxiolytic effects.

An important question raised by these findings is whether substance P antagonists and established antidepressant drugs really act via distinct molecular targets (for example, monoamine transporters or the NK_1 receptor). We investigated whether markers of monoamine function were changed after acute administration of the substance P antagonist L-760,735 in vivo. In gerbils, treatment with reserpine caused hypothermia and ptosis. Consistent with the results of previous studies using mice (*26*), phenelzine and imipramine reversed these effects, whereas fluoxetine and L-760,735 did not (all test compounds administered at 30 mg/kg). Moreover, repeated administration of a substance P antagonist for 14 days did not cause down-regulation of cortical β-adrenoreceptors in rats (*27*). These findings show that central substance P receptor blockade does not augment norepinephrine function in a manner resembling the action of monoamine oxidase inhibitors or tricyclic antidepressants.

The ability of L-760,735 to potentiate 5-HT–mediated behaviors (*28*) was compared with that of other antidepressant drugs in gerbils. Administration of the 5-HT precursor 5-hydroxytryptophan in animals pretreated with pargyline (100 mg/kg intraperitoneally) caused a behavioral syndrome comprising wet dog shakes, forepaw treading, splaying of the hindlimbs, and flattened body posture. The frequency or number of animals exhibiting these behaviors was increased by administration of monoamine reuptake inhibitors as compared with animals receiving vehicle, but not in animals treated with L-760,735 (all compounds tested at 30 mg/kg). Consistent with these findings, acute central substance P receptor blockade did not affect extracellular 5-HT concentration, as measured by in vivo microdialysis in the rat hippocampus (*29*).

Thus, L-760,735 did not augment norepinephrine or serotonin function in the manner seen with established antidepressant drugs. The atypical profile of L-760,735 in these assays supports the proposal that the antidepressant activity of substance P antagonists is mediated via a novel mechanism, as was also reflected by the absence of sexual dysfunction, nausea, or other adverse effects associated with established antidepressant drugs in patients. Because many clinically used antidepressant drugs have relatively poor pharmacological specificity, the possibility that their therapeutic effects might be explained through a direct blockade of central substance P receptors was examined. A range of structurally diverse antidepressant drugs (phenelzine, imipramine, fluoxetine, mianserin, reboxetine) was found to have no significant affinity for human, guinea pig, and gerbil NK_1 receptors (IC_{50} > 10 μM) (*13*).

Possible CNS sites for antidepressant activity of substance P antagonists. There are many potential CNS sites that might mediate the antidepressant activity of substance P antagonists and other classes of antidepressant drugs. Of these, the amygdala in particular has been implicated as a potential site for the action of established antidepressant drugs. Thus, focal injection of imipramine into the amygdala produces effects in assays involving psychological stress resembling those seen after systemic administration (*30*).

A major output projection from the amygdala is to the hypothalamus. In cats, electrical stimulation of the amygdala facilitates the emergence of a defensive rage syndrome elicited by stimulation of the hypothalamus. Imipramine and related antidepressants block attack behavior caused by hypothalamic stimulation in cats (*31*). Substance P provides a powerful monosynaptic input from the medial amygdala to the hypothalamus that is important for the expression of defensive rage in cats. Thus, both systemic and intrahypothalamic infusion of the substance P antagonist CP-96,345 blocks the facilitatory effects of amygdaloid stimulation on defensive rage (*32*). A second major projection from the amygdala is to the periaqueductal gray (PAG), where electrical stimulation also causes defensive rage behavior in cats. The PAG shows dense immunoreactivity for substance P, and the amount of preprotachykinin mRNA has been shown to increase in the dorsal PAG after social defeat stress in rats (*33*). These and other substance P–containing pathways may therefore be important in the response to stressors, particularly in relation to vocalization responses examined in the present studies using guinea pigs.

After activation, substance P NK_1 receptors are internalized and at least 1 hour elapses before the protein is recycled into the neuronal membrane (*34*). We exploited this internalization as a way to map those brain regions in which release of endogenous substance P occurred after maternal separation of guinea pig pups (*35*). We saw an increase (about 60%) in the number of cells showing NK_1 receptor internalization in the anterior-basolateral amygdala after maternal separation for 5 min (*36*) (Fig. 4). We conclude that psychological stress causes release of sub-

Table 1. Incidence (≥5%) of clinical adverse experiences (AEs) in patients receiving MK-869 (300 mg) or paroxetine (20 mg). Only those AEs observed at rates numerically greater than with placebo are shown.

AE	Percentage of patients		
	MK-869 (n = 71)	Paroxetine (n = 72)	Placebo (n = 70)
Nervous system and psychiatric			
Headache	32	28	24
Somnolence	20	19	9
Insomnia	11	14	9
Irritability	7	1	0
Nervousness	1	6	4
Digestive			
Nausea	18	29*	10
Diarrhea	11	15	9
Dry mouth	9	8	7
Flatulence	7	3	4
Dizziness	7	8	6
Anorexia	4	11	3
Respiratory			
Upper respiratory infection	6	8	3
Skin and appendages			
Sweating	3	11	3
Urogenital			
Total sexual dysfunction: combined terms	3†	26*	4
Libido decreased	0	6	0
General sexual dysfunction	0†	8*	0
Ejaculation disorder (% males)	3†	20	7
Impotence (% males)	3	10	4
General			
Asthenia/fatigue	14	19*	4
Abdominal pain	9	6	3

*Paroxetine greater than placebo, $p \leq 0.05$. †MK-869 less than paroxetine, $p \leq 0.05$.

stance P in the amygdala. It is not yet known whether stress-induced NK_1 receptor internalization is inhibited by substance P antagonists; however, this possibility is strongly suggested by the prevention of substance P–induced receptor internalization in the striatum of rats by the NK_1 receptor antagonist RP 67580 (*34*).

Conclusions. MK-869, a brain-penetrant substance P antagonist, represents an innovative mechanistic approach to antidepressant therapy. The precise mechanism by which these therapeutic effects are brought about is not yet known, as is the case for traditional antidepressant therapies, but preclinical evidence suggests that it may involve the integration of emotional responses to stress by brain structures such as the amygdala. The possibility that alterations in substance P or the NK_1 receptor are primarily involved in the pathogenesis of depression requires further investigation, which may lead to a better understanding of the pathophysiology of this disease.

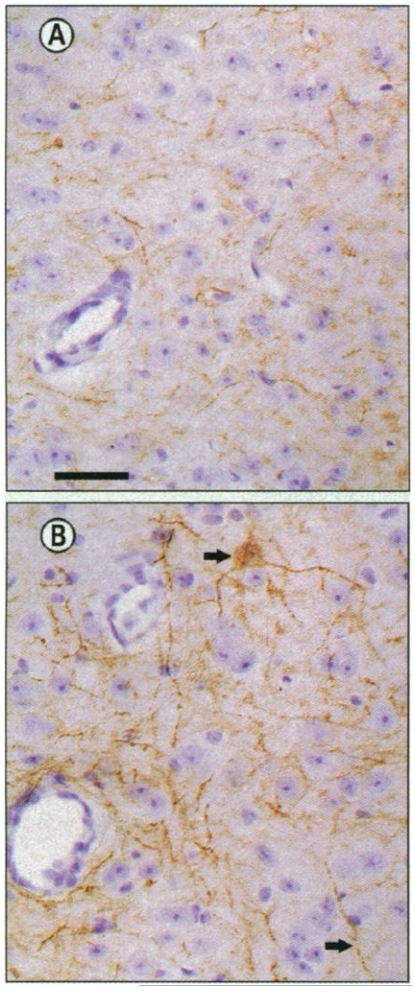

Fig. 4. Immunocytochemical demonstration of NK_1 receptor endocytosis in anterior-basolateral amygdala in response to maternal separation of guinea pig pups. (**A**) In nonisolated guinea pig pups, NK_1 receptor immunoreactivity (IR) is associated with the somatic and dendritic surfaces of the neurons. (**B**) After maternal separation for 5 min, there was a marked transfer of NK_1 receptor IR from the cell surface to intracellular endosomes, accompanied by structural reorganization of dendrites characterized by varicosities rich in NK_1 receptor–positive endosomes and linked by thin fibers (arrows). Scale bar, 50 μM. ANOVA revealed an increase ($p \leq 0.016$) in the number of cells in the anterior-basolateral amygdala exhibiting NK_1 receptor endocytosis in animals separated from their mothers compared with nonisolated pups (n = 5 or 6).

References and Notes

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19. The drug washout period was 7 ± 3 days except for fluoxetine (4 weeks) and monoamine oxidase inhibitors (2 weeks). Chloral hydrate (500 to 1000 mg daily) could be prescribed sparingly for insomnia during the study, but this was not to be taken within 24 hours before clinic visits. All other psychotropic medications were prohibited.
20. All medications were to be taken orally, once daily in the evening, for 6 weeks. MK-869 (three 100-mg tablets per dose, with matching placebo capsules to paroxetine) and encapsulated paroxetine 20-mg tablets (with matching placebo to MK-869) were used in a double-dummy design. Paroxetine tablets were placed in opaque capsules to maintain the blind; these had comparable stability and dissolution to the unencapsulated form.
21. The primary efficacy analysis compared mean changes from baseline between MK-869 and placebo on HAM-D21 total score at week 6 using an "all patients treated, last observation carried forward" approach. For a two-tailed test with p = 0.05, the power to detect a 4-point difference of change (a clinically significant effect) between MK-869 and placebo was 84% (based on standard deviation of 8.0). Pairwise comparisons of MK-869

or paroxetine versus placebo were performed at each week by analysis of variance (ANOVA) with treatment group and investigative site included as factors. Statistical testing at early time points was performed post hoc and was not adjusted for multiplicity. Similar analyses evaluated secondary efficacy variables. Pairwise comparisons of clinical AEs, or discontinuations for AEs, used Fisher's exact test.

22. Seventy-one patients were treated with MK-869 (300 mg/day), 72 with paroxetine (20 mg/day), and 70 with placebo. About 70% of the patients in each group completed the full 6-week course of therapy. The demonstration of antidepressant efficacy of MK-869 was not confounded by different rates of discontinuation in the treatment groups. The treatment × site effect was significant (ANOVA, $p = 0.031$). The interactions were not qualitative between any two treatment groups. Therefore, the ANOVA model with treatment and site was applied for treatment comparisons. See *Science* Online (www.sciencemag.org) for details on demographics and baseline comparability, an accounting of patients who completed or discontinued from the study, and statistics on interaction of treatment × investigative site (with respect to mean change from baseline to week 6 in HAM-D21 total scores).
23. See *Science* Online (www.sciencemag.org) for details on mean changes from baseline in Hamilton Depression Scale factors with MK-869, paroxetine, and placebo, with pairwise comparison p values for MK-869 and paroxetine versus placebo.
24. Mild transaminase elevations (1.5 to 2.5 times the upper limit of normal) were observed in three patients receiving MK-869 and caused discontinuation. High titers of Epstein-Barr antibodies (immunoglobulin G) and clinical signs of viral infection were observed in two of these patients; in the third, AST was mildly elevated on entry into the study. One patient was discontinued on paroxetine for elevated transaminases. Mild transient increases in transaminases (AST, ALT, or both) were regarded as laboratory AEs in two additional patients on MK-869, four on paroxetine, and one on placebo.
25. See *Science* Online (www.sciencemag.org) for details on mean changes from baseline in HAM-D21 data ("as observed" approach) with MK-869, paroxetine, and placebo, with pairwise comparison p values for MK-869 and paroxetine versus placebo.
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37. We thank D. Slaughter, C. Assang, M. Beer, S. Patel, C. Barton, P. H. Hutson, F. Kelleher, M. Rigby, S. Boyce, and R. Shigemoto for their contributions to the preclinical studies.

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