Current Biology, Vol. 15, 1961–1967, November 8, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.09.043

The HMG-CoA Reductase Inhibitor Lovastatin Reverses the Learning and Attention Deficits in a Mouse Model of Neurofibromatosis Type 1

Weidong Li,[1,2,3,4] Yijun Cui,[1,2,3,4] Steven A. Kushner,[1,2,3,4] Robert A.M. Brown,[1,2,3,4] J. David Jentsch,[3,4] Paul W. Frankland,[1,2,3,4,5] Tyrone D. Cannon,[2,3,4] and Alcino J. Silva[1,2,3,4,*]

[1]Department of Neurobiology
[2]Neuropsychiatric Institute
[3]Department of Psychology
[4]Brain Research Institute
University of California, Los Angeles
Los Angeles, California 90095

Summary

Neurofibromatosis Type 1 (NF1) is a common neurological disorder caused by mutations in the gene encoding Neurofibromin, a p21Ras GTPase Activating Protein (GAP) [1]. Importantly, NF1 causes learning disabilities and attention deficits [2, 3]. A previous study showed that the learning and memory deficits of a mouse model of NF1 (*nf1*$^{+/-}$) appear to be caused by excessive p21Ras activity leading to impairments in long-term potentiation (LTP) [4], a cellular mechanism of learning and memory [5–7]. Here, we identify lovastatin as a potent inhibitor of p21Ras/Mitogen Activated Protein Kinase (MAPK) activity [8, 9] in the brain. Lovastatin is a specific inhibitor of three-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, used commonly for the treatment of hypercholesterolemia [10]. We report that lovastatin decreased the enhanced brain p21Ras-MAPK activity of the *nf1*$^{+/-}$ mice, rescued their LTP deficits, and reversed their spatial learning and attention impairments. Therefore, these results demonstrate that lovastatin may prove useful in the treatment of Neurofibromatosis Type 1.

Results and Discussion

The key pathophysiologic mechanism underlying NF1 mutations in both mice [4, 11–13] and humans [14, 15] is increased p21Ras activity. Therapeutic interventions designed to inhibit p21Ras function have been proposed as treatments of NF1 [16]. Posttranslational farnesylation is required for the membrane localization and function of p21Ras, and farnesylation provides a potential target for NF1 pharmacotherapy [17]. Indeed, pharmacologic inhibitors of farnesyltransferase downregulate p21Ras activity. It is unknown, however, whether any of these inhibitors have the in vivo pharmacokinetics, biodistribution, and safety profile required for the long-term treatment of cognitive dysfunction in NF1 [18].

Lovastatin, a specific inhibitor of the rate-limiting enzyme in cholesterol biosynthesis (HMG-CoA reductase), is widely used to treat hyperlipidemia in humans [19]. Previous studies have shown that lovastatin can inhibit p21Ras isoprenylation and activity [8, 20]. Since the cognitive deficits caused by mutations in the NF1 gene may result from increased p21Ras activity, we hypothesized that lovastatin could rescue these deficits.

The NF1 null mutation mouse is lethal as a homozygote but is viable as a heterozygote [13]. Interestingly, we have not detected neuroanatomic deficits in the *nf1*$^{+/-}$ mice, although they do display a range of behavioral abnormalities that parallel the cognitive profile associated with NF1 patients [1].

We analyzed the effect of lovastatin treatment on p21Ras/MAPK by using Western blotting. *nf1*$^{+/-}$ mice and wild-type littermate controls (wt) were injected with 10 mg/kg lovastatin subcutaneously once per day for 4 days, and they were sacrificed on the 4th day, 6 hr after the final injection. Levels of p44/42 were examined in both cortex and hippocampus by SDS-PAGE. Proteins were transferred to membranes and hybridized with anti-phospho p44/42 MAPK (Cell Signaling) antibody. The results in Figure 1A show that the levels of phosphorylated p44/42 MAPK are higher in both the cortex ($p < 0.05$) and hippocampus ($p < 0.05$) of *nf1*$^{+/-}$ mice compared to wt. Several days of treatment with 10 mg/kg of lovastatin decreased the levels of phosphorylated p44/42 MAPK in *nf1*$^{+/-}$ mice, resulting in roughly equal amounts of phosphorylated p44/p42 MAPK as wt (cortex: $p = 0.759$, hippocampus: $p = 0.850$). Sample loading was controlled by reprobing the nitrocellulose membranes used for the analysis with an anti-p44/42 MAPK antibody.

Neurofibromin functions as a p21Ras GTPase-activating protein that catalyzes the conversion of active GTP bound p21Ras to the inactive GDP bound form. We therefore examined the impact of lovastatin treatment on p21Ras activity directly. Cortical and hippocampal extracts were reacted with GST-Raf1-RBD beads (Pierce Bio), which specifically bind p21Ras-GTP, the active form of p21Ras. p21Ras-GTP was resolved by SDS-PAGE and visualized with an anti-pan p21Ras antibody (Sigma). Again, p21Ras-GTP levels were elevated in the *nf1*$^{+/-}$ compared to wt for both cortex ($p < 0.05$) and hippocampus ($p < 0.05$). Lovastatin treatment decreased the levels of p21Ras-GTP in both the cortex and hippocampus of *nf1*$^{+/-}$ to the level of wt mice (cortex: $p = 0.789$, hippocampus: $p = 0.195$) (Figure 1B). This result is consistent with decreased levels of MAPK activity that we found in *nf1*$^{+/-}$ mice. Altogether, these data demonstrate that lovastatin can decrease p21Ras/MAPK activity in the cortex and hippocampus and may therefore be useful to treat cognitive deficits of the *nf1*$^{+/-}$ mice.

NF1 in humans is associated with a broad spectrum of cognitive profiles, which may include learning disabilities, attentional disorders, and altered visuospatial skills [2, 3, 21, 22]. We examined the effect of lovastatin on *nf1*$^{+/-}$ mice in a variety of behavioral and cognitive tests to assess how the loss of NF1 impacted on these behaviors and whether lovastatin could rescue any observed deficits.

*Correspondence: silvaa@mednet.ucla.edu
[5]Present address: Department of Integrative Biology, Hospital for Sick Children Research Institute, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.

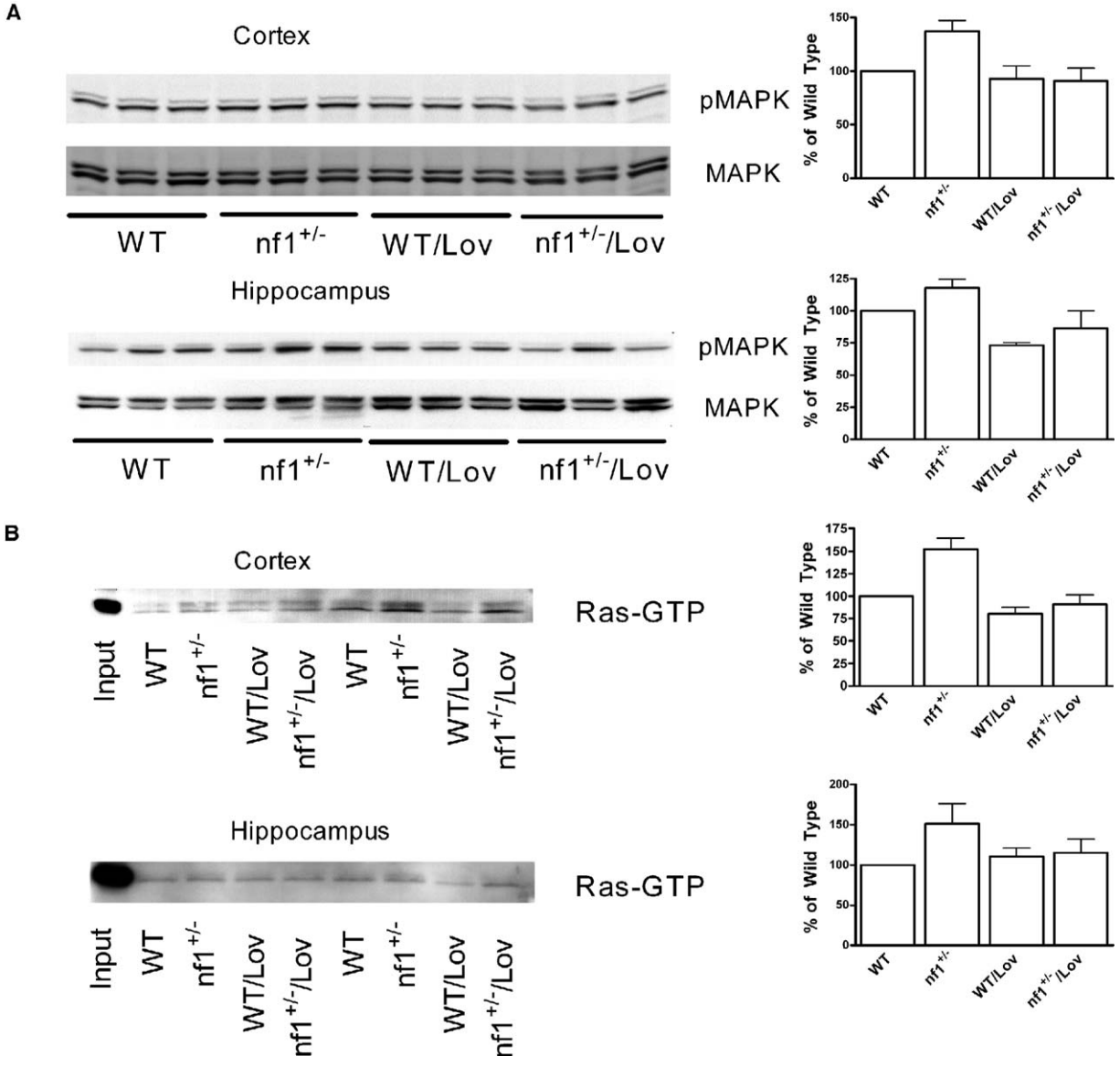

Figure 1. Downregulation of p21Ras-MAPK Activity in *nf1*$^{+/-}$ Mice by Lovastatin

(A) Lovastatin was effective at decreasing active MAPK in the *nf1*$^{+/-}$ mice (4–9 mice per group).

(B) Lovastatin also decreased active p21Ras (p21Ras-GTP) in *nf1*$^{+/-}$ mice (6–7 mice for each group). Error bars represent ± one standard error.

To investigate altered attentional function in the *nf1*$^{+/-}$ mice, we used the lateralized reaction-time task, a test that measures divided visuospatial attention and is dependent upon prefrontal cortex. Animals nosepoke to trigger the delivery of a visual target stimulus on one side of their visual field. The spatial location and time of onset of the target is unpredictable and so attention must be distributed across space and time. The task difficulty is altered by changing the duration that the target stimulus is visible. Wild-type and *nf1*$^{+/-}$ mice were tested with lovastatin (*nf1*$^{+/-}$ = 7, wt = 7) or placebo (*nf1*$^{+/-}$ = 14, wt = 10). The rate of correct responses (an index of attention accuracy) revealed a genotype × treatment × target stimulus duration interaction (ANOVA, $F_{2,70} = 3.200$, $p < 0.05$). The correct response rate of wt mice was significantly higher than that of *nf1*$^{+/-}$ mice (PLSD, $p < 0.05$; Figure 2A) at the most difficult stimulus duration (0.5 s), indicating that the *nf1*$^{+/-}$ mice have impaired attentional function.

In contrast, the performance of *nf1*$^{+/-}$ mice treated with lovastatin was indistinguishable from that of wt mice at a target stimulus duration of 0.5 s (PLSD, $p = 0.148$; Figure 2B) and was significantly higher than *nf1*$^{+/-}$ given placebo (PLSD, $p < 0.05$). These data demonstrate that *nf1*$^{+/-}$ mice exhibit substantial attention deficits and that lovastatin treatment can reverse these deficits.

We have previously shown that *nf1*$^{+/-}$ mice have abnormal spatial learning tested in the hidden version of the water maze [4, 23], a task that is sensitive to lesions

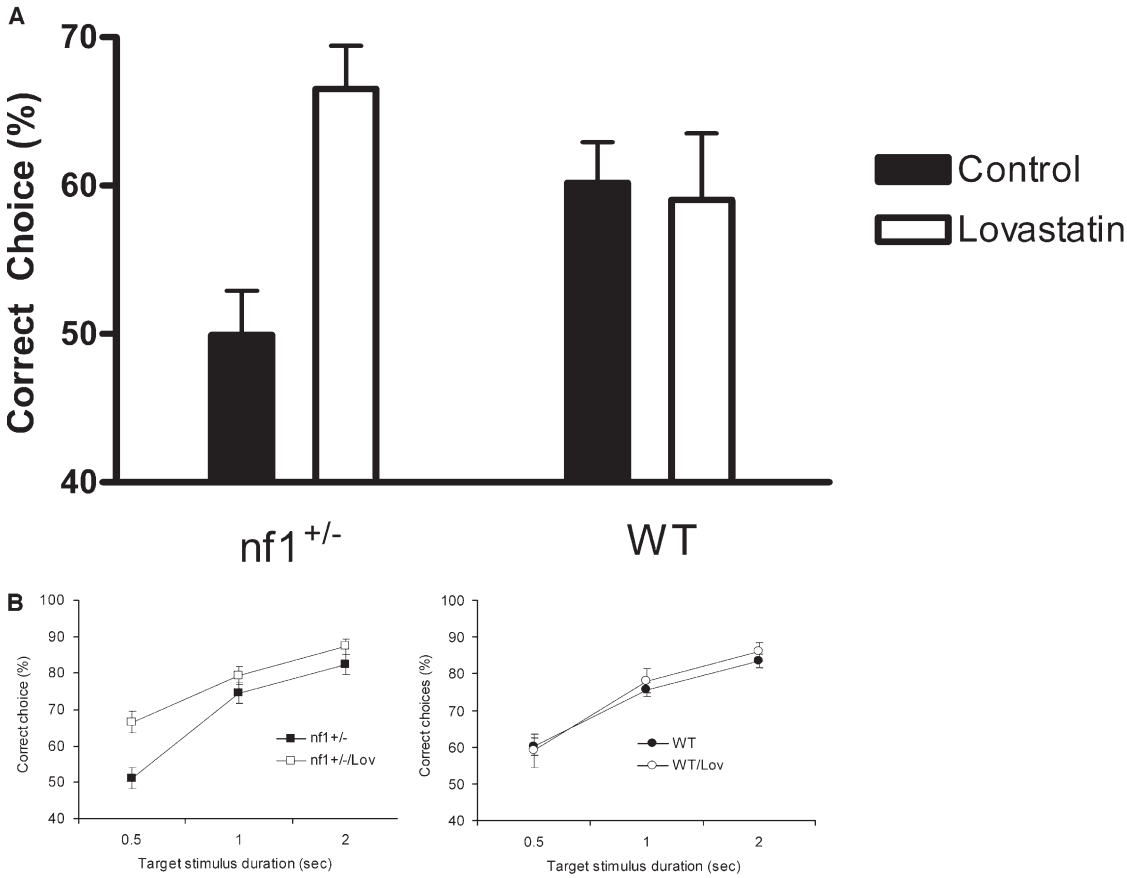

Figure 2. Rescue by Lovastatin of Attention Deficits in *nf1*$^{+/-}$ Mice

(A) *nf1*$^{+/-}$ mice have deficits compared to wt animals in the 0.5 ms target stimulus duration condition of the lateralized reaction time test. The deficit is rescued by lovastatin treatment.

(B) The deficit is seen only at the most difficult interval. Longer intervals produce equivalent performance between the groups (wt = 10, *nf1*$^{+/-}$ = 14, wt with lovastatin = 7, *nf1*$^{+/-}$ with lovastatin = 7). Error bars represent ± one standard error.

of the hippocampus and multiple cortical sites including retrosplenial, orbitofrontal, medial prefrontal cortices, and the cingulum bundle [24–27]. To test the hypothesis that lovastatin can rescue the deficits of *nf1*$^{+/-}$ mice in this task, we injected 10 mg/kg lovastatin subcutaneously for 3 days before the first training day, and then 6 hr before behavioral training daily. Mice were trained with two trials per day. No differences were observed between genotypes and/or treatment groups in measures of acquisition, floating, thigmotaxic behavior, or swimming speed (data not shown).

Spatial learning was assessed in probe trials given at the end of water maze training on days 5 and 7, since previous studies have shown that probe trial performance is the most faithful measure of spatial learning in the Morris maze [28]. In the probe trials, the platform was removed from the pool and the mice were allowed to search for it for 60 s. Neither genotype had learned the task by the day 5 probe (Figure 3A). Differences emerged between the genotypes and treatments (ANOVA, $F_{1,82} = 4.415$, $p < 0.05$) after 2 more days of training. Wild-type mice spent significantly more time searching in the target quadrant than *nf1*$^{+/-}$ mice (PLSD, $p < 0.05$; Figure 3B), confirming that the *nf1*$^{+/-}$ mutants have impaired spatial learning. In contrast, the mutants treated with lovastatin spent as much time as wt in the target quadrant ($p = 0.862$; Figure 3B) and significantly more time than mutants given placebo ($p < 0.05$). These results demonstrate that the spatial learning deficits of the *nf1*$^{+/-}$ mice are not caused by irreversible developmental abnormalities since they are reversed with acute lovastatin treatment in adult mutant mice.

Previous studies report a high incidence of attention-deficit hyperactivity disorder (ADHD) in NF1 patients and support an association between ADHD and learning problems in these children [2, 29]. Children with ADHD are reported to have significantly reduced prepulse inhibition (PPI) [30]. PPI assays sensory "gating" of environmental stimuli. A sudden acoustic stimulus will normally elicit a whole-body startle response. If the startle-producing stimulus is preceded by a weak prestimulus, the startle response is inhibited in normal persons and animals [31]. We tested whether *nf1*$^{+/-}$ mice have deficits in this task and whether these deficits could also be reversed by lovastatin treatment (as described above). A two-way repeated measures ANOVA revealed significant main effects of genotype

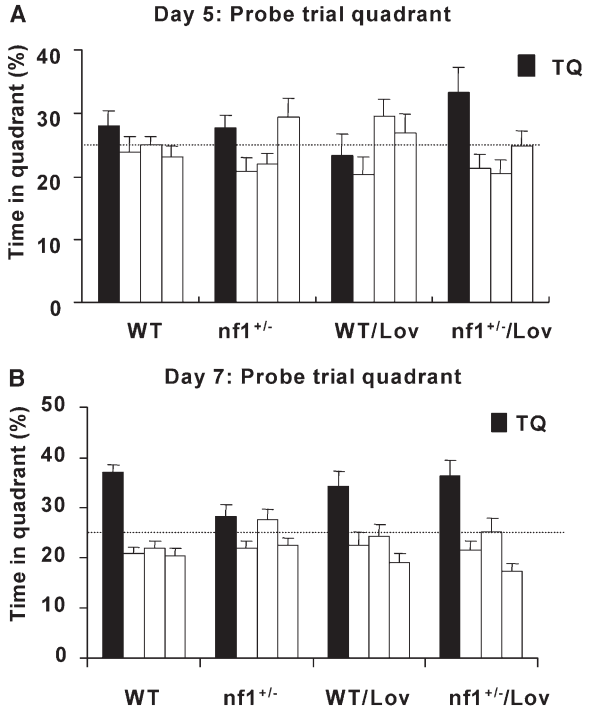

Figure 3. Lovastatin Rescue of Spatial Learning Deficits in *nf1*$^{+/-}$ Mice

(A) Percent time spent in each quadrant during a water maze probe trial on day 5.

(B) Percent time spent in each quadrant during a probe trial on day 7. Quadrants are target quadrant (TQ), adjacent left, opposite quadrant (OP), and adjacent right (wt = 24, *nf1*$^{+/-}$ = 21, wt with lovastatin = 21, *nf1*$^{+/-}$ with lovastatin = 20). Error bars represent ± one standard error.

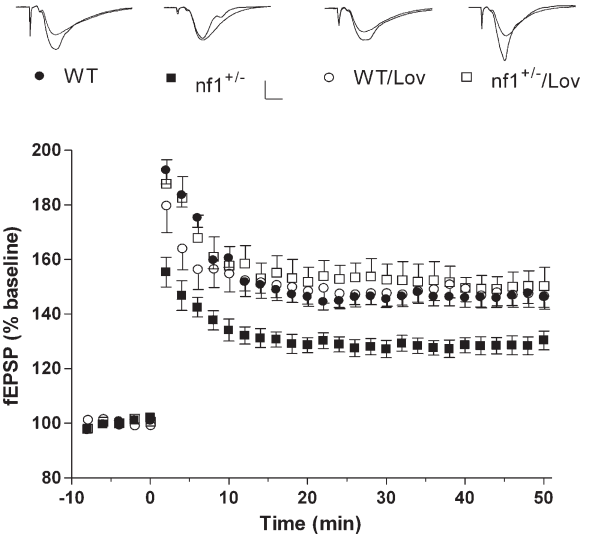

Figure 5. Rescue by Lovastatin of *nf1*$^{+/-}$ Deficits in Long-Term Potentiation

Percentage of baseline fEPSP is plotted over time. A five theta-burst induction protocol was delivered at time 0 (wt = 8, *nf1*$^{+/-}$ = 7, wt with lovastatin = 8, *nf1*$^{+/-}$ with lovastatin = 7). For clarity purposes, error bars (standard error of the mean) are shown in only one direction. Representative traces are shown from left to right: wt off drug, *nf1*$^{+/-}$ off drug, wt on lovastatin, *nf1*$^{+/-}$ on lovastatin. Horizontal bar represents 2 ms. Vertical bar represents 0.5 mV.

and treatment (Figure 4). The *nf1*$^{+/-}$ animals have deficient PPI ($F_{1,30} = 7.42$, $p < 0.05$), and lovastatin treatment resulted in an increase in performance ($F_{1,30} = 6.61$, $p < 0.05$). Importantly, the performance of *nf1*$^{+/-}$ animals on lovastatin is indistinguishable from that of wt animals on placebo (PLSD, $p = 0.877$), demonstrating that lovastatin can reverse the PPI deficits of these mutants. The increased PPI of the wt animals on lovastatin may be attributed to Ras inhibition. Current models of PPI attribute the mechanism to a presynaptic inhibition of release [32]. Given that Ras activity is known to increase presynaptic release of transmitter [33], the effects of lovastatin and PPI would work in concert to decrease presynaptic release. In the case of the *nf1*$^{+/-}$ mutants, which have upregulated Ras function, the decrease merely brings them back into the normal range. In wt animals, release would be greatly curtailed, resulting in the enhancement of PPI.

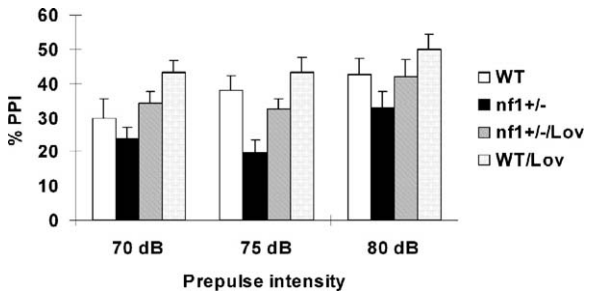

Figure 4. Lovastatin Rescues Sensory Gating Deficits as Measured with PPI

PPI was examined via prepulses at three different stimulus intensities (70, 75, and 80 dB) (wt = 8, *nf1*$^{+/-}$ = 8, wt with lovastatin = 9, *nf1*$^{+/-}$ with lovastatin = 9). Error bars represent ± one standard error.

We have previously shown that the learning deficits of the *nf1*$^{+/-}$ mice are likely caused by impairments in LTP [4], a stable long-lasting change in synaptic strength widely believed to be a key cellular mechanism for learning and memory that is dependent upon Ras/MAPK function [5–7, 34]. We therefore determined whether the LTP deficits in *nf1*$^{+/-}$ mice could be reversed by lovastatin. Mice were injected with 10 mg/kg of lovastatin as described above. We examined LTP in hippocampal slices at the Schaffer collateral/CA1 synapse, since LTP at this synapse has served as a model system for associative changes in synaptic strength between neurons [5–7]. LTP was measured after a 5 theta-burst stimulation protocol (TBS, five bursts 200 ms apart, each burst of 4 pulses at 100 Hz), which mimics in vivo activity of hippocampal neurons during exploratory behavior [35]. Figure 5 shows that there was a difference among the genotypes and treatments (ANOVA, $F_{1,26} = 8.55$, $p < 0.05$). The LTP measured in *nf1*$^{+/-}$ mutants was significantly lower than in wt mice (PLSD, $p < 0.05$; Figure 5), a result consistent with previously published findings [4]. The amount of LTP induced in *nf1*$^{+/-}$ mutants treated with lovastatin was sig-

nificantly higher than that induced in mutants (PLSD, $p < 0.05$; Figure 5) and equivalent to that of wt (PLSD, $p = 0.602$; Figure 5). These data demonstrate that the lovastatin treatment completely reversed the LTP deficits of the *nf1*$^{+/-}$ mice.

The present results demonstrate that lovastatin treatment can reverse the biochemical, electrophysiological, and cognitive deficits observed in a mouse model of NF1 and that these deficits are not due to irreversible developmental changes. Previous studies have shown that an increase in p21Ras activity is central to the pathophysiology associated with NF1 [16], and our biochemical data demonstrate that lovastatin reverses the abnormally elevated p21Ras/MAPK activity in an animal model of NF1. Importantly, our studies demonstrated that the dose of lovastatin that is effective in *nf1*$^{+/-}$ mice did not affect cognitive function in control mice, a result consistent with randomized studies performed with human subjects that did not identify a reliable effect of lovastatin on cognitive function [36, 37]. Nevertheless, it is worth noting that there are sporadic reports that statins can be associated with mild cognitive impairment [38]. Altogether, the studies reported here demonstrate that the cognitive deficits associated with NF1 can be reversed by treatments with lovastatin, a widely prescribed drug that is known to be well tolerated even in long-term treatments. Thus, these data suggest that lovastatin could be used to treat the cognitive impairments associated with Neurofibromatosis Type 1 in humans.

Experimental Procedures

Animal Experiments

All animal protocols were approved by the Chancellor's Animal Research Committee at the University of California at Los Angeles, in accordance with the National Institutes of Health guidelines. Studies were performed in 129T2/SvEmsJ-C57BL/6 F1 hybrids generated by an F1 cross between *nf1*$^{+/-}$ mice (maintained in the C57BL/6 background for more than 11 generations) and wild-type mice on the 129T2/SvEmsJ background with wild-type littermates used as controls. All experiments were carried out blind with respect to genotype and treatment.

Lovastatin Solution and Pellet

Because of the extended nature of the lateralized reaction time task (see below), lovastatin was administered orally as pellets, while in other tasks lovastatin was injected in the lactone form. Mevinolin (lovastatin, Sigma) was dissolved in ethanol, then incubated at room temperature in 1N NaOH and water to mevinolin to the sodium salt. The final mevinolin solution (4 mg/ml) was adjusted to pH 7.5 with HCl [10, 39]. Pellets for oral administration were prepared by mixing crushed lovastatin tablets (Eon Labs) (prescription formulation) with melted peanut butter chips (H.B. Reese Candy Co.). The mixture was molded into 200 mg pellets containing 0.15 mg Lovastatin and were administered to mice once daily.

Western Blot Analysis for p44/42 MAP Kinase Phosphorylation and p21Ras Activity

Cortical and hippocampal regions from 4 to 7 control and lovastatin-treated mice were isolated and homogenized in protein extraction buffer. Supernatant was collected after centrifugation, and products were separated by electrophoresis on a 4%–15% gradient or 12% SDS-PAGE (Bio-Rad Laboratories). Gels were blotted to nitrocellulose membranes and then blocked for 1 hr at room temperature with Tris-buffered saline (TBS) containing 0.1% (v/v) Tween-20 and 5% (w/v) nonfat dry milk. After washing in TBST, membranes were hybridized at room temperature with anti-phospho-p44/42 (Cell Signaling) antibody diluted 1:1000 in the blocking solution. The membranes were visualized with the ECL Plus protocol (Amersham BioSciences) and quantified by a Storm System phosphor imager (Amersham Biosciences). Membranes were stripped in stripping buffer and reprobed with anti-p44/42 (Cell Signaling) as a control for protein loading. The amount of phosphorylated p44/42 was expressed as a ratio of total p44/42. Other groups were normalized to the wt ratio.

For p21Ras activity assay, p21Ras pull-down experiments were performed with the EZ-detect p21Ras activation kit (Pierce Biotechnology), according to the manufacturer's protocol. 25 μl of sample per lane was loaded on SDS-PAGE. p21Ras-GTP was detected and quantified by Western blotting as described via an anti-pan-p21Ras antibody (Sigma). The ratio of Ras-GTP in each group was normalized to wt.

Lateralized Reaction Time Task

Animals (placebo-treated: *nf1*$^{+/-}$ = 14, wt = 10; lovastatin treated: *nf1*$^{+/-}$ = 7, wt = 7) were food deprived to 90% of their free-feeding weights. Mice were trained in a miniaturized versions of a "5-choice" box (Med Associates, St Albans, VT). The chamber was equipped with a horizontal array of five apertures that could be internally illuminated and the opposite wall was fitted with a pellet magazine for reward delivery. Animals were shaped to produce a "poke and hold" response in the central aperture during which a side aperture would be illuminated. A correct response was scored when the animals correctly poked the aperture that had been illuminated. Animals that did not perform at 75% accuracy at 1 s target stimulus duration during the training phase were dropped. Mice were tested on a variable duration condition in which the target aperture was illuminated for 0.5, 1.0, or 2.0 s (varied within session). We measured correct responses/total trials, which vary as a function of the target stimulus duration and was therefore analyzed by use of stimulus duration as a repeated measure [40].

Water Maze

The basic protocol for the water maze experiments has been previously described [11]. Mice from the 129T2/SvEmsJ-C57B/6 F1 genetic background were given two trials per day (30 s intertrial intervals) with a probe trial (60 s) at the end of training days 5 and 7. Mice were given subcutaneous injections of 10 mg/kg lovastatin or vehicle for 3 days before the first training day and then 6 hr before training every day.

Prepulse Inhibition

Mice were food deprived as described above. 5 min after placement in the conditioning chamber, mice were presented with 20 noise bursts (40 ms duration, 120 dB, <1 ms rise/fall time). In the prepulse inhibition phase, mice were presented with a total of 90 trials (30 trials each at 70, 75, and 80 dB). For each prepulse intensity (20 ms duration, <1 ms rise/fall time), there were three types of trial: prepulse alone, prepulse/startle stimulus, and startle stimulus alone. In the prepulse/startle stimulus trial, the onset of the prepulse preceded the onset of the startle stimulus by 100 ms. Background noise levels were maintained at 68 dB throughout testing, and the trials were spaced 15 s [41].

Hippocampal LTP

Transverse hippocampal slices (400 mm thick) were placed in a submerged recording chamber perfused (2 ml/min) with ACSF containing 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 at 34°C. Field excitatory postsynaptic potentials (fEPSPs) were recorded with a Pt/Ir electrode (FHC, Bowdoinham, ME) from the stratum radiatum layer of the area CA1 elicited by stimulation of the Schaffer collateral/commissural afferents by two bipolar electrodes placed 300 μm on either side of the recording electrode. Test pulses of 60 μA alternated between the two electrodes every minute throughout the duration of the experiment. After 20 min of stable baseline, LTP was induced via a TBS protocol as described in the text delivered to the test pathway. Multiple slices from a single animal were averaged and entered into analysis as a single subject.

The responses from the last 10 min block of recordings (40–50 min) were compared for statistical tests.

Statistical Analysis

Western blots were analyzed with the one-sample t test (mean = 100, one tail upper). We analyzed percent time in training quadrant for the different genotypes from the water maze by using 2-way ANOVA. Attention data was analyzed with three-way repeated-measures ANOVA on the average of correct response rate. PPI data was analyzed with two-way repeated-measures ANOVA. For the electrophysiological experiments, the significance of differences between the groups was determined by two-way ANOVA. Post-hoc comparisons (Fisher's PLSD) between groups were carried out where appropriate.

Acknowledgements

We thank R. Chen, A. Matynia, and M.J. Sanders for technical assistance. We are grateful to R.M. Costa for invaluable discussions of the experiment described here. This work was supported by grants from the NIH (R01 NS38480), Neurofibromatosis, Inc. (National, Illinois, Mass Bay Area, Minnesota, Arizona, Kansas and Central Plains, Mid-Atlantic, and Texas chapters), the Merck, and the National Neurofibromatosis Foundation (NNF) to A.J.S. Y.C. was supported by a Young Investigator Award from NNF.

Received: May 24, 2005
Revised: August 24, 2005
Accepted: September 19, 2005
Published: November 7, 2005

References

1. North, K. (2000). Neurofibromatosis type 1. Am. J. Med. Genet. *97*, 119–127.
2. Koth, C.W., Cutting, L.E., and Denckla, M.B. (2000). The association of neurofibromatosis type 1 and attention deficit hyperactivity disorder. Neuropsychol. Dev. Cogn. Sect. C Child Neuropsychol. *6*, 185–194.
3. Ozonoff, S. (1999). Cognitive impairment in neurofibromatosis type 1. Am. J. Med. Genet. *89*, 45–52.
4. Costa, R.M., Federov, N.B., Kogan, J.H., Murphy, G.G., Stern, J., Ohno, M., Kucherlapati, R., Jacks, T., and Silva, A.J. (2002). Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. Nature *415*, 526–530.
5. Kandel, E.R. (2001). The molecular biology of memory storage: a dialog between genes and synapses. Biosci. Rep. *21*, 565–611.
6. Nakazawa, K., McHugh, T.J., Wilson, M.A., and Tonegawa, S. (2004). NMDA receptors, place cells and hippocampal spatial memory. Nat. Rev. Neurosci. *5*, 361–372.
7. Silva, A.J. (2003). Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. J. Neurobiol. *54*, 224–237.
8. Sebti, S.M., Tkalcevic, G.T., and Jani, J.P. (1991). Lovastatin, a cholesterol biosynthesis inhibitor, inhibits the growth of human H-ras oncogene transformed cells in nude mice. Cancer Commun. *3*, 141–147.
9. Xu, X.Q., McGuire, T.F., Blaskovich, M.A., Sebti, S.M., and Romero, G. (1996). Lovastatin inhibits the stimulation of mitogen-activated protein kinase by insulin in HIRcB fibroblasts. Arch. Biochem. Biophys. *326*, 233–237.
10. Kita, T., Brown, M.S., and Goldstein, J.L. (1980). Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in livers of mice treated with mevinolin, a competitive inhibitor of the reductase. J. Clin. Invest. *66*, 1094–1100.
11. Costa, R.M., Yang, T., Huynh, D.P., Pulst, S.M., Viskochil, D.H., Silva, A.J., and Brannan, C.I. (2001). Learning deficits, but normal development and tumor predisposition, in mice lacking exon 23a of Nf1. Nat. Genet. *27*, 399–405.
12. Brannan, C.I., Perkins, A.S., Vogel, K.S., Ratner, N., Nordlund, M.L., Reid, S.W., Buchberg, A.M., Jenkins, N.A., Parada, L.F., and Copeland, N.G. (1994). Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. Genes Dev. *8*, 1019–1029.
13. Jacks, T., Shih, T.S., Schmitt, E.M., Bronson, R.T., Bernards, A., and Weinberg, R.A. (1994). Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. Nat. Genet. *7*, 353–361.
14. Basu, T.N., Gutmann, D.H., Fletcher, J.A., Glover, T.W., Collins, F.S., and Downward, J. (1992). Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. Nature *356*, 713–715.
15. DeClue, J.E., Papageorge, A.G., Fletcher, J.A., Diehl, S.R., Ratner, N., Vass, W.C., and Lowy, D.R. (1992). Abnormal regulation of mammalian p21ras contributes to malignant tumor growth in von Recklinghausen (type 1) neurofibromatosis. Cell *69*, 265–273.
16. Weiss, B., Bollag, G., and Shannon, K. (1999). Hyperactive Ras as a therapeutic target in neurofibromatosis type 1. Am. J. Med. Genet. *89*, 14–22.
17. Yan, N., Ricca, C., Fletcher, J., Glover, T., Seizinger, B.R., and Manne, V. (1995). Farnesyltransferase inhibitors block the neurofibromatosis type I (NF1) malignant phenotype. Cancer Res. *55*, 3569–3575.
18. Mazieres, J., Pradines, A., and Favre, G. (2004). Perspectives on farnesyl transferase inhibitors in cancer therapy. Cancer Lett. *206*, 159–167.
19. Corvol, J.C., Bouzamondo, A., Sirol, M., Hulot, J.S., Sanchez, P., and Lechat, P. (2003). Differential effects of lipid-lowering therapies on stroke prevention: a meta-analysis of randomized trials. Arch. Intern. Med. *163*, 669–676.
20. Mendola, C.E., and Backer, J.M. (1990). Lovastatin blocks N-ras oncogene-induced neuronal differentiation. Cell Growth Differ. *1*, 499–502.
21. Brewer, V.R., Moore, B.D., 3rd, and Hiscock, M. (1997). Learning disability subtypes in children with neurofibromatosis. J. Learn. Disabil. *30*, 521–533.
22. Schrimsher, G.W., Billingsley, R.L., Slopis, J.M., and Moore, B.D., 3rd. (2003). Visual-spatial performance deficits in children with neurofibromatosis type-1. Am. J. Med. Genet. *120A*, 326–330.
23. Silva, A.J., Frankland, P.W., Marowitz, Z., Friedman, E., Lazlo, G., Cioffi, D., Jacks, T., and Bourtchuladze, R. (1997). A mouse model for the learning and memory deficits associated with neurofibromatosis type I. Nat. Genet. *15*, 281–284.
24. Harker, K.T., and Whishaw, I.Q. (2004). Impaired place navigation in place and matching-to-place swimming pool tasks follows both retrosplenial cortex lesions and cingulum bundle lesions in rats. Hippocampus *14*, 224–231.
25. Mogensen, J., Moustgaard, A., Khan, U., Wortwein, G., and Nielsen, K.S. (2005). Egocentric spatial orientation in a water maze by rats subjected to transection of the fimbria-fornix and/or ablation of the prefrontal cortex. Brain Res. Bull. *65*, 41–58.
26. Steffenach, H.A., Witter, M., Moser, M.B., and Moser, E.I. (2005). Spatial memory in the rat requires the dorsolateral band of the entorhinal cortex. Neuron *45*, 301–313.
27. Vafaei, A.A., and Rashidy-Pour, A. (2004). Reversible lesion of the rat's orbitofrontal cortex interferes with hippocampus-dependent spatial memory. Behav. Brain Res. *149*, 61–68.
28. Brandeis, R., Brandys, Y., and Yehuda, S. (1989). The use of the Morris Water Maze in the study of memory and learning. Int. J. Neurosci. *48*, 29–69.
29. Mautner, V.F., Kluwe, L., Thakker, S.D., and Leark, R.A. (2002). Treatment of ADHD in neurofibromatosis type 1. Dev. Med. Child Neurol. *44*, 164–170.
30. Castellanos, F.X., Fine, E.J., Kaysen, D., Marsh, W.L., Rapoport, J.L., and Hallett, M. (1996). Sensorimotor gating in boys with Tourette's syndrome and ADHD: preliminary results. Biol. Psychiatry *39*, 33–41.
31. Swerdlow, N.R., Braff, D.L., and Geyer, M.A. (1999). Cross-species studies of sensorimotor gating of the startle reflex. Ann. N Y Acad. Sci. *877*, 202–216.
32. Nusbaum, M.P., and Contreras, D. (2004). Sensorimotor gating: startle submits to presynaptic inhibition. Curr. Biol. *14*, R247–R249.

33. Seeger, G., Yan, L., Gartner, U., Huemmeke, M., Barmashenko, G., Mittmann, T., Heumann, R., and Arendt, T. (2004). Activation of Ras in neurons modifies synaptic vesicle docking and release. Neuroreport *15*, 2651–2654.
34. Orban, P.C., Chapman, P.F., and Brambilla, R. (1999). Is the Ras-MAPK signalling pathway necessary for long-term memory formation? Trends Neurosci. *22*, 38–44.
35. Larson, J., Wong, D., and Lynch, G. (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res. *368*, 347–350.
36. Gengo, F., Cwudzinski, D., Kinkel, P., Block, G., Stauffer, L., and Lines, C. (1995). Effects of treatment with lovastatin and pravastatin on daytime cognitive performance. Clin. Cardiol. *18*, 209–214.
37. Muldoon, M.F., Barger, S.D., Ryan, C.M., Flory, J.D., Lehoczky, J.P., Matthews, K.A., and Manuck, S.B. (2000). Effects of lovastatin on cognitive function and psychological well-being. Am. J. Med. *108*, 538–546.
38. Wagstaff, L.R., Mitton, M.W., Arvik, B.M., and Doraiswamy, P.M. (2003). Statin-associated memory loss: analysis of 60 case reports and review of the literature. Pharmacotherapy *23*, 871–880.
39. Feleszko, W., Mlynarczuk, I., Balkowiec-Iskra, E.Z., Czajka, A., Switaj, T., Stoklosa, T., Giermasz, A., and Jakobisiak, M. (2000). Lovastatin potentiates antitumor activity and attenuates cardiotoxicity of doxorubicin in three tumor models in mice. Clin. Cancer Res. *6*, 2044–2052.
40. Jentsch, J.D. (2003). Genetic vasopressin deficiency facilitates performance of a lateralized reaction-time task: altered attention and motor processes. J. Neurosci. *23*, 1066–1071.
41. Frankland, P.W., Wang, Y., Rosner, B., Shimizu, T., Balleine, B.W., Dykens, E.M., Ornitz, E.M., and Silva, A.J. (2004). Sensorimotor gating abnormalities in young males with fragile X syndrome and Fmr1-knockout mice. Mol. Psychiatry *9*, 417–425.