Age-dependent decrease of synaptic plasticity in the neocortex of αCaMKII mutant mice

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ABSTRACT Synaptic long-term potentiation (LTP) and long-term depression (LTD) were studied in the visual cortex of mutant mice lacking α-calcium/calmodulin-dependent protein kinase II (αCaMKII). In adult mutants, little LTD or LTP could be elicited using standard conditioning protocols. However, substantial LTD and LTP were induced in 4- to 5-week-old mutants. Thus, the reduction in cortical plasticity in αCaMKII (−/−) mice is conditional, with the relevant condition being postnatal age.

Mice lacking the α subunit of calcium/calmodulin-dependent protein kinase II (CaMKII) reportedly lack long-term potentiation (LTP) and long-term depression (LTD) in adult hippocampus (area CA1) (1, 2), and have a severe deficit in experience-dependent plasticity in adult somatosensory cortex (3). However, experience-dependent plasticity is only partially impaired in the immature visual cortex (4), and is apparently unaffected in 4- to 8-week-old somatosensory cortex (3), of the same mutant animals. On the surface, these findings appear to challenge the hypothesis that the mechanisms of LTD and LTP are employed for experience-dependent receptive field modifications during neocortical development (5–7). However, it is unknown to what extent LTD and LTP in the sensory neocortex are sensitive to the genetic ablation of αCaMKII, especially in young animals. Here we report experiments designed to examine this question. These data were first reported at the 1994 Society for Neuroscience meeting in Miami.

MATERIALS AND METHODS

αCaMKII "knockout" mice were prepared as described (1). These mice are in short supply due to breeding difficulties. For our studies, a total of 13 αCaMKII (−/−) mice and 12 wild-type controls were available. Of these, six mutants were 4–5 weeks of age, and seven were adults (>6 months old); six controls were 4–5 weeks of age, and six were adults. In addition, we studied four heterozygous littermates that were 4–5 weeks of age.

All experiments were performed blind to the genotype of the animals. Brain slice preparation was performed as described (8). Slices of visual cortex were maintained in an atmosphere of humidified 95% O_2 and 5% CO_2, and superfused with 30°C artificial cerebrospinal fluid (ACSF) at a rate of 1 ml/min. The ACSF was saturated with 95% O_2 and 5% CO_2, and contained 124 mM NaCl/5 mM KCl/1.25 mM NaH_2PO_4/1 mM $MgSO_4$/2 mM $CaCl_2$/26 mM $NaHCO_3$/10 mM dextrose. A site in the middle of the cortical thickness, confirmed histologically to correspond to layer IV and upper layer V, was stimulated to

evoke field potentials (FPs) in layer III, as described (8). The amplitude of the maximum negative FP in layer III was used as a measure of the evoked population excitatory synaptic current. Changes in the amplitude of the maximum negative FP reflect changes in the magnitude of a synaptic current sink (9), and correlate with changes in the initial slope of excitatory postsynaptic potentials recorded intracellularly in layer III neurons (8, 10). Baseline responses were obtained every 15 sec with a stimulation intensity that yielded a half-maximal response. The duration of the baseline was at least 10 min for LTP experiments, and at least 20 min for LTD experiments. Theta-burst stimulation (TBS) was used to induce LTP. TBS consists of brief bursts of stimuli delivered every 200 msec, with each burst containing four pulses at 100 Hz. Nine hundred pulses were delivered at 1 Hz to induce LTD.

Only data from slices with stable recordings (<5% change over the baseline period) were included in the analysis of LTP and LTD. The data from these slices were then assigned to one of five experimental groups following the genotyping of the animals: young homozygous mutant, young heterozygous mutant, young wild-type control, adult homozygous mutant, and adult wild-type control. The group data were then analyzed as follows: (*i*) the maximum negative FP amplitude data for each experiment were expressed as percentages of the preconditioning baseline average, (*ii*) the time scale in each experiment was converted to time from the onset of conditioning, and (*iii*) the time-matched, normalized data were averaged across experiments and expressed as the means (±SEM). Within each group, the statistical significance of a change produced by conditioning stimulation was assessed with a paired *t* test, comparing values immediately before conditioning stimulation with those 20 min after TBS or 30 min after 1 Hz stimulation. Young and old, mutant and control groups were compared at the time point 20 min after cessation of TBS and 30 min after 1 Hz stimulation using a two-way ANOVA. Cumulative histograms were also constructed to show the data from each slice in each experimental group.

RESULTS

LTP in Sensory Neocortex of αCaMKII Mutants and Controls. Layer III field potentials, representing population excitatory postsynaptic responses, were measured in response to electrical stimulation at a site corresponding to layer IV and upper layer V (hereafter referred to as "layer IV" stimulation). As reported previously for rats and cats (11), TBS of layer IV resulted in robust and stable LTP of layer III field potentials in control mouse visual cortex. In adult wild-type mice, the response 20 min after conditioning stimulation was 126.5 ± 4.0% of the pretetanus baseline value (mean ± SEM; *n* = 25 slices from 6

Abbreviations: LTP, long-term potentiation; LTD, long-term depression; αCaMKII, α-calcium/calmodulin-dependent protein kinase II; TBS, theta-burst stimulation; FP, field potential.
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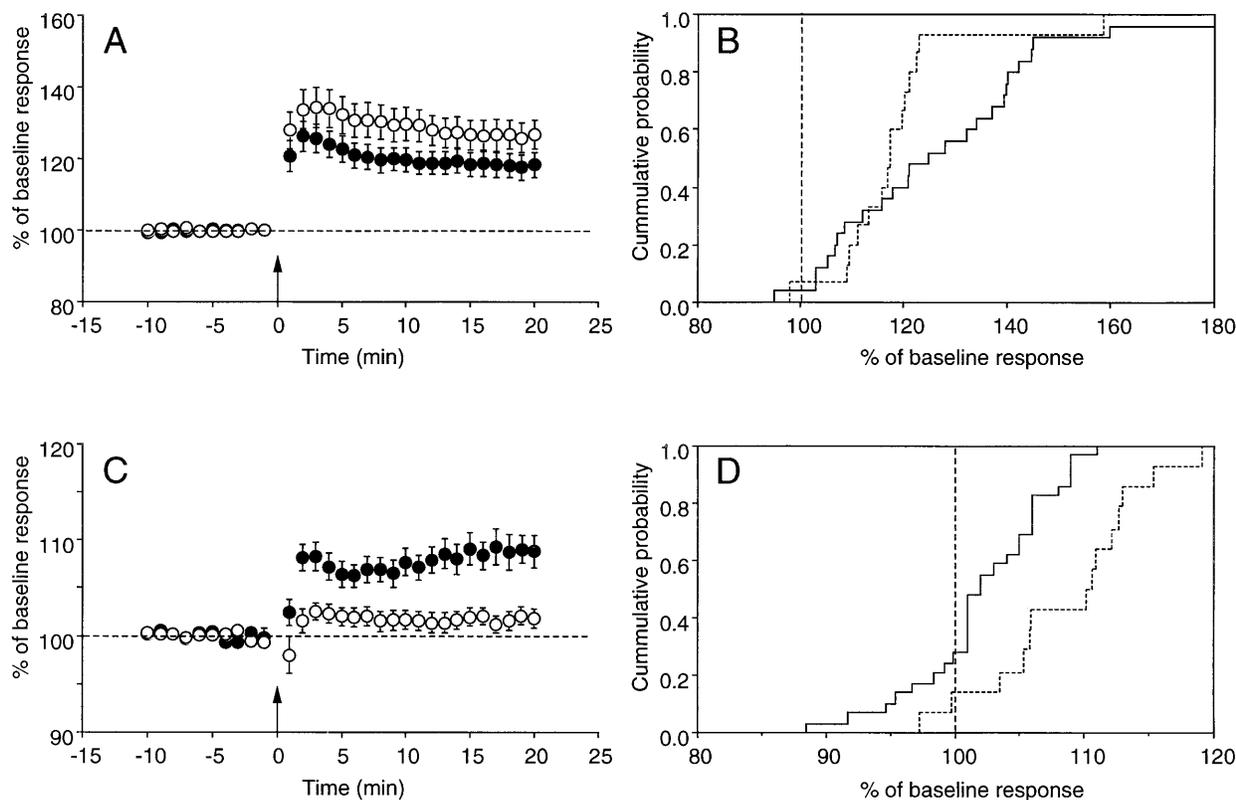
FIG. 1.   LTP in young and old, wild-type and αCaMKII($-/-$) visual cortex. (*A*) The effects of TBS of layer IV (indicated by the upward arrow) on synaptic field potentials in layer III of wild types. Each point represents the mean response ($\pm$SEM) normalized to the average baseline value. Open circles, data from adults ($n = 25$ slices); solid circles, data from 4- to 5-week-old mice ($n = 15$ slices). (*B*) Cumulative histograms showing responses 20 min after TBS in all wild-type slices from adult (solid line) and young (dashed line) animals. (*C*) The effects of TBS on layer III field potentials in slices from αCaMKII($-/-$) mice. Open circles, data from adult mutants ($n = 29$ slices); solid circles, data from 4- to 5-week-old mutants ($n = 14$ slices). Note that the scales of the y-axes differ in *A* and *C*. (*D*) Cumulative histograms showing responses 20 min after TBS in all αCaMKII($-/-$) slices from adult (solid line) and young (dashed line) animals.

mice; Fig. 1*A*, open circles; Fig. 1*B*, solid line). Average LTP was of similar magnitude in slices prepared from 4- to 5-week-old wild types, measuring $118.2 \pm 3.4\%$ ($n = 15$ slices from 6 animals; Fig. 1*A*, solid circles; Fig. 1*B*, dashed line). Examples of LTP of field potentials in visual cortical slices from young and adult animals are shown in Fig. 2 *A* and *B*.

In αCaMKII($-/-$) mice from both age-groups, there was a clear deficit in LTP. However, the severity of the deficit appeared to be age-dependent. In adult mice homozygous to the αCaMKII mutation, TBS produced a response measuring only $102.1 \pm 1.0\%$ of baseline ($n = 29$ slices from 7 mice; Fig. 1*C*, open circles; Fig. 1*D*, solid line). However, in 4- to 5-week-old mutants, TBS consistently yielded synaptic potentiation, averaging $108.7 \pm 1.6\%$ ($n = 14$ slices from 6 mice; Fig. 1*C*, solid circles; Fig. 1*D*, dashed line). Examples of the effect of TBS on field potentials in slices from young and old αCaMKII($-/-$) animals are shown in Fig. 2 *C* and *D*.

Two-way ANOVA showed a significant interaction between the effects of age and genotype on LTP [$F(3,79) = 6.195, P < 0.02$]. Tests of simple effects demonstrated that the magnitude of LTP in mutants and wild types was significantly different in adult animals [$F(1,52 = 47.026, P < 0.0002$], but was not in young animals [$F(1,27) = 3.8, P > 0.05$]. Thus, the effects of the genetic ablation of αCaMKII on LTP are significantly more severe in adult animals than in young animals.

LTP was also investigated in 4- to 5-week-old animals heterozygous to the αCaMKII mutation. These animals showed a phenotype that was identical to the homozygous mutants ($108.9 \pm 1.1\%$; $n = 12$ slices from 4 mice).

LTD in Sensory Neocortex of αCaMKII Mutants and Controls. We sought to investigate LTD in adult mice by

delivering 900 stimuli at 1 Hz, a procedure that has been shown to be effective for inducing homosynaptic depression in rat hippocampus (12, 13), visual cortex (10), somatosensory cortex, and motor cortex (14). Unfortunately, we found that the LTD produced in adult mouse visual cortex using this procedure was of very small magnitude (Fig. 3*A*, open circles; Fig. 3*B*, solid line). The response 30 min after 1 Hz stimulation was $97.9 \pm 1.9\%$ in adult wild types ($n = 5$ slices from 3 mice). Similarly, following induction of LTP ($136.2 \pm 6.5\%$; $n = 7$ slices from 3 mice), 1 Hz stimulation produced little depotentiation ($95.4 \pm 3.7\%$ of the potentiated level). However, as described, in the CA1 region of rat hippocampus (15, 16) and in layer IV of cat visual cortex (17), greater LTD could be elicited in young animals. In 4- to 5-week-old wild-type mice, LTD measured $84.6 \pm 3.6\%$ of baseline ($n = 11$ slices from 5 mice; Fig. 3*A*, solid circles; Fig. 3*B*, dashed line).

Similar to the adult wild-type controls, 1 Hz stimulation produced little LTD in adult αCaMKII($-/-$) animals ($95.7 \pm 1.4\%, n = 7$ slices from 5 mice; Fig. 3*C*, open circles; Fig. 3*D*, solid line). However, robust LTD was observed in visual cortex of 4- to 5-week-old mutant mice ($89.4 \pm 2.0\%$ of baseline, $n = 15$ slices from 6 mice; Fig. 3*C*, solid symbols; Fig. 3*D*, dashed line).

Two-way ANOVA showed a significant effect of age on LTD [$F(1,34) = 10.65, P < 0.003$], but no effect of genotype, and no interaction between age and genotype. Young animals heterozygous to the αCaMKII mutation also showed LTD at levels similar to those observed in the homozygous mutant and wild-type animals ($87.5 \pm 2.4\%$ of baseline, $n = 9$ slices from 4 mice).

DISCUSSION

Fig. 4 summarizes the deficits in synaptic plasticity in visual cortex of mice lacking αCaMKII. In 4- to 5-week-old mutants, the
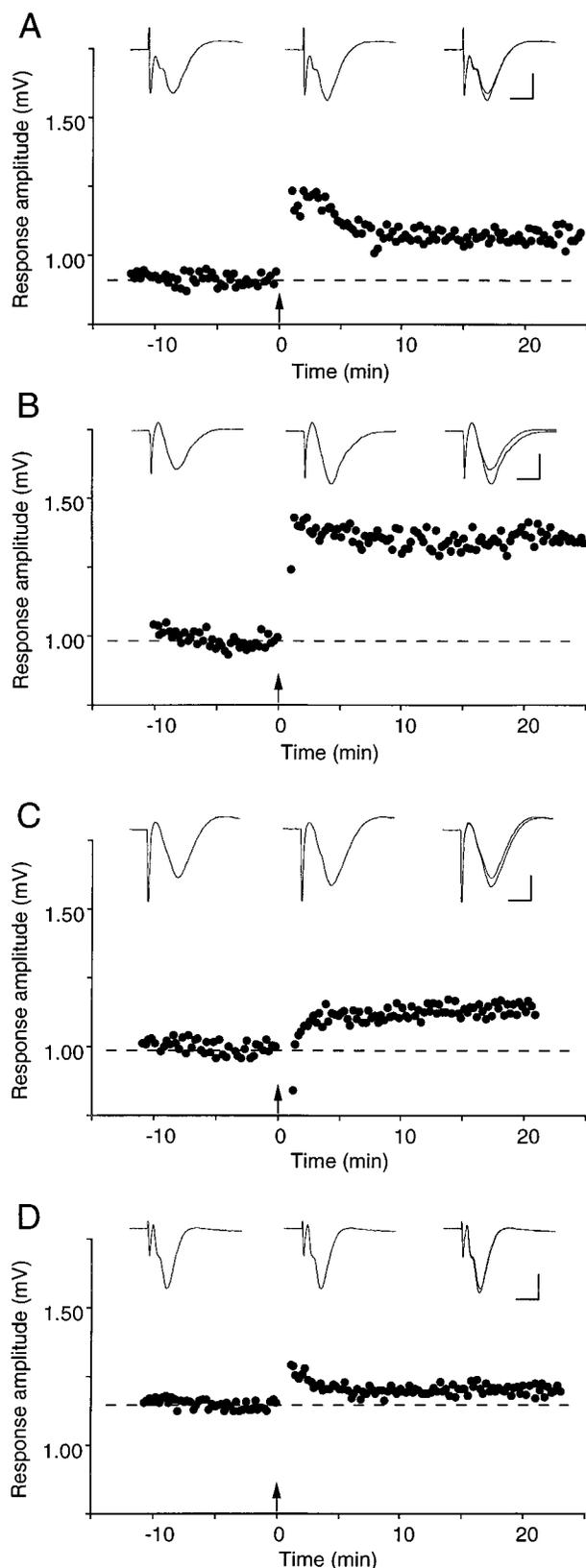
FIG. 2.   Examples of the effects of TBS (upward arrows) in visual cortex of wild-type and αCaMKII($-/-$) mice. (*A*) 4- to 5-week-old wild type. (*B*) Adult wild type. (*C*) 4- to 5-week old mutant. (*D*) Adult mutant. Individual traces are FPs recorded before and 20 min after TBS; they are superimposed on the right. (Bars = 0.5 mV, 5 ms.)

magnitude of LTP, although less than control, is still half the wild-type value (Fig. 4*A*). The magnitude of LTD is greater than

two-thirds the control value in young animals (Fig. 4*B*). These data show that in the sensory neocortex of young animals, LTP and LTD are not eliminated by the genetic ablation of αCaMKII. Similarly, it was reported very recently that LTP is reduced only by about 50% in the hippocampus of young αCaMKII knockouts (18). We therefore conclude that LTD and LTP can occur independently of αCaMKII in young animals.

It could be argued that LTP measuring only 109% of baseline in young mutants, while statistically significant, is of little or no biological significance. Of course, the same question could be raised for control LTP (measuring 118% in wild-type visual cortex), especially since the biological significance of LTP has not been established in any system. Nonetheless, it seems a reasonable possibility that a mechanism capable of altering synaptic strength by 9% over the course of only a few minutes could make a major contribution to experience-dependent plasticity over the course of postnatal development.

The roughly 50% decrease in LTP magnitude in the visual cortex of young mutants is interesting in light of recent data from Gordon *et al.* (4), who studied ocular dominance plasticity in αCaMKII knockout mice. They found that visual cortical receptive fields develop normally in the mutants, but that there is an approximately 50% reduction in ocular dominance plasticity in 4- to 5-week-old animals. Detailed analysis revealed, however, that this average effect was explained by the fact that half the animals showed normal plasticity and half showed virtually no plasticity. Although our sample of young mutants is small (n = 6 mice), we do not see similar animal-to-animal variability in our data. If the data from slices derived from the same mouse are averaged to yield an individual mean value for each animal, we find that individual mean LTP never exceeded 113%, but was greater than 106% in five of the six mutants studied.

The LTP deficit in adult mutants is far more severe than it is in young animals (Fig. 4*C*). However, the data do not allow us to conclude that LTP is absent in adult αCaMKII mutants. First, we have not exhausted all the possible methods for inducing LTP. Second, even with the method we used, the very small increase in the response following TBS (102.1 $\pm$ 1.0%) was consistent across experiments and therefore achieved statistical significance ($P < 0.01$, paired t test). Nonetheless, we can say that theta-burst-induced LTP is very different in immature and adult mutants.

Some important questions remain open. For example, is the LTP that remains in the mutants N-methyl-D-aspartate (NMDA)-receptor-dependent? Is LTP affected even less by the mutation in animals less than 4 weeks? Unfortunately, due to breeding difficulties, αCaMKII mutant mice are currently unavailable for further experimentation. However, although NMDA-receptor-independent LTP has been reported in cat visual cortex (19), the means of induction and the time course are very different from what we describe here. Previous work has shown that theta-burst-induced LTP, which exhibits a rapid onset, is reliably blocked by NMDA-receptor antagonists (8). In regard to the second question, we note that the late development of αCaMKII in neocortex virtually ensures that any plasticity in neonates will be independent of this molecule. Indeed, NMDA-receptor-dependent LTP has been observed in mouse neocortex at ages less than postnatal day 4 (20). In rat neocortex, αCaMKII mRNA is barely detectable at this age (21).

Our data suggest that as mice lacking αCaMKII grow older, they undergo a conditional "knockout," not of a molecule, but of a process: synaptic plasticity. In mutant animals, the mechanisms of LTD and LTP apparently are available during the postnatal development of sensory neocortex, but are then greatly reduced in adults. Remarkably, in the same mutants, experience-dependent receptive field plasticity is also intact in layer III of somatosensory cortex in 4- to 8-week-old animals, but is absent in adults (3). Moreover, in wild-type mice,
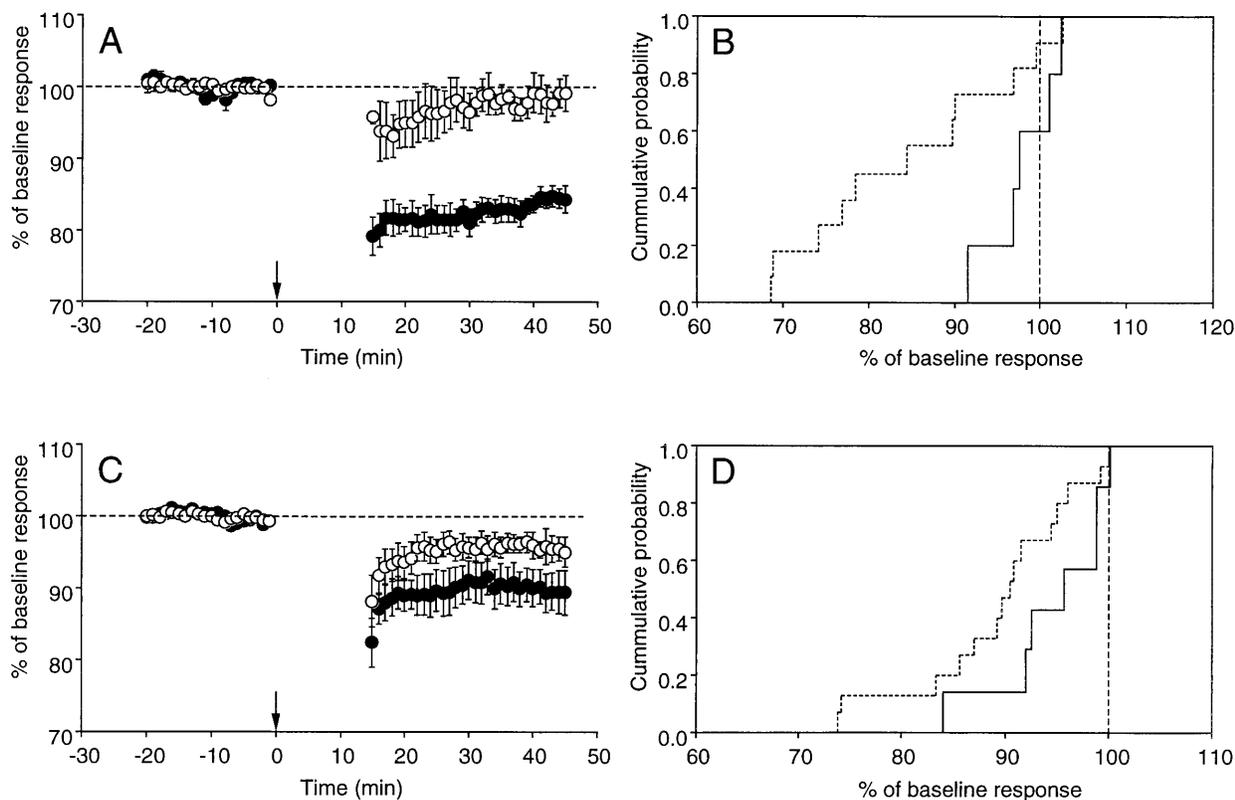
FIG. 3. LTD in young and old, wild-type and αCaMKII(−/−) visual cortex. (*A*) The effects of 1 Hz stimulation (900 pulses) of layer IV (indicated by the downward arrow) on synaptic field potentials in layer III of wild types. Open circles, data from adults (*n* = 5 slices); solid circle, data from 4- to 5-week-old mice (*n* = 11 slices). (*B*) Cumulative histograms showing responses 30 min after 1 Hz stimulation in all wild-type slices from adult (solid line) and young (dashed line) animals. (*C*) The effects of 1 Hz stimulation on layer III field potentials in slices from αCaMKII(−/−) mice. Open circles, data from adult mutants (*n* = 7 slices); solid circles, data from 4- to 5-week-old mutants (*n* = 15 slices). (*D*) Cumulative histograms showing responses 30 min after 1 Hz stimulation in all αCaMKII(−/−) slices from adult (solid line) and young (dashed line) animals.

sensory-deprivation-induced depression of cortical responses declines with postnatal age in a manner that closely corresponds to that which we observe for LTD. Thus, there is a good correlation between the effects of age and genetic mutation on LTD, LTP, and receptive field plasticity, strengthening the hypothesis that the mechanisms of LTD and LTP are used for naturally occurring synaptic modifications in the superficial layers of sensory neocortex.
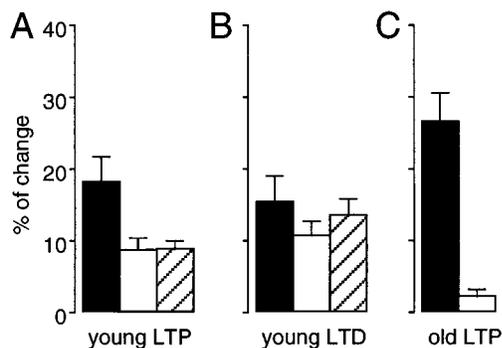
FIG. 4. Comparison of synaptic plasticity in visual cortex of wild-type (solid bars; +/+), homozygous mutant (open bars; −/−), and heterozygous mutant (striped bars; +/−) mice. (*A*) LTP produced by theta-burst stimulation in 4- to 5-week-old animals. (*B*) LTD produced by 1 Hz stimulation in 4- to 5-week-old animals. (*C*) LTP produced by theta-burst stimulation in adult animals.

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