MHC class I modulates NMDA receptor function and AMPA receptor trafficking

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Proteins of the major histocompatibility complex class I (MHCI) are known for their role in immunity and have recently been implicated in long-term plasticity of excitatory synaptic transmission. However, the mechanisms by which MHCI influences synaptic plasticity remain unknown. Here we show that endogenous MHCI regulates synaptic responses mediated by NMDA-type glutamate receptors (NMDARs) in the mammalian central nervous system (CNS). The AMPA/NMDA ratio is decreased at MHCI-deficient hippocampal synapses, reflecting an increase in NMDAR-mediated currents. This enhanced NMDAR response is not associated with changes in the levels, subunit composition, or gross subcellular distribution of NMDARs. Increased NMDAR-mediated currents in MHCI-deficient neurons are associated with characteristic changes in AMPA receptor trafficking in response to NMDAR activation. Thus, endogenous MHCI tonically inhibits NMDAR function and controls downstream NMDAR-induced AMPA receptor trafficking during the expression of plasticity.

immune | GluR | hippocampus

Proteins of the major histocompatibility complex class I (MHCI) are best known for their role in adaptive immunity, but several lines of evidence suggest they also have nonimmune functions in neurons (1, 2). MHCI is expressed by healthy neurons in the developing and adult CNS (3–7). Neuronal MHCI mRNA levels are dynamic during development and are regulated by electrical activity (3, 4) and by the cAMP-response element-binding protein (CREB) (8). MHCI protein is enriched in synaptic fractions (4) and is detected in hippocampal dendritic spines, where it colocalizes with PSD-95 (9).

Studies in mice genetically deficient for cell-surface MHCI ($\beta2m^{-/-}TAP^{-/-}$ mice) suggest a role for MHCI in activity-dependent plasticity. In MHCI-deficient mice, NMDA receptor (NMDAR)-dependent hippocampal long-term potentiation (LTP) is enhanced, whereas long-term depression (LTD) is abolished (4). Although the mechanisms by which MHCI mediates immune signaling have been relatively well characterized, nothing is known about how MHCI contributes to NMDAR-dependent plasticity in vitro or in vivo.

In the adult hippocampus, plasticity induced by activation of NMDARs is expressed as changes in the trafficking and function of AMPA receptors (AMPARs) (10–13). In current models, the magnitude and kinetics of NMDAR activation determine whether potentiation or depression is induced, with large, transient NMDAR activation causing LTP and smaller, longer-lasting activation causing LTD (14, 15). Therefore, to better understand the role of endogenous MHCI in the induction or expression of synaptic plasticity, we examined the levels, distribution, trafficking, and function of AMPA- and NMDA-type receptors in MHCI-deficient hippocampal neurons.

The current experiments reveal an unexpected role for postsynaptic MHCI in controlling NMDAR function. Loss of MHCI causes a drop in the AMPA/NMDA ratio and an enhancement of NMDAR-mediated responses at CA3–CA1 synapses. This enhancement cannot be attributed to changes in the levels, subunit composition, or gross subcellular distribution of NMDARs. The increase in basal NMDAR-mediated responses in MHCI-deficient neurons is not associated with a change in basal AMPAR properties but is associated with changes in the trafficking of AMPARs in response to NMDA. Thus, in addition to its immune role, MHCI restricts NMDAR function and controls downstream NMDAR-induced AMPAR trafficking.

Results

Basal AMPAR- and NMDAR-Mediated Synaptic Responses. To test if MHCI affects the induction of plasticity by modifying basal glutamatergic transmission, whole-cell voltage-clamp recordings were performed at Schaffer collateral/CA1 synapses in acute hippocampal slices from WT or MHCI-deficient ($\beta2m^{-/-}TAP^{-/-}$; *Materials and Methods*) animals. AMPAR-mediated responses decay rapidly after reaching their peak, whereas NMDAR-mediated responses decay over a longer time course. These differential decay kinetics were used to determine the proportion of the excitatory postsynaptic current (EPSC) mediated by AMPARs versus NMDARs (*Materials and Methods*). At $\beta2m^{-/-}TAP^{-/-}$ synapses, the AMPA/NMDA ratio was significantly lower than at WT synapses (Fig. 1*A*; WT 2.0 ± 0.1, $n = 15$ cells; $\beta2m^{-/-}TAP^{-/-}$ 1.5 ± 0.1, $n = 12$ cells; $^*P < 0.05$, two-tailed unpaired t test). Similar results were obtained when NMDAR-mediated currents were isolated by pharmacologically blocking AMPARs (Fig. S1).

The lower AMPA/NMDA ratio in MHCI-deficient neurons could reflect an increase in the NMDAR-mediated response and/or a decrease in the AMPAR-mediated response. To distinguish among these possibilities, we performed extracellular recordings and plotted the input–output (I/O) relationship for pharmacologically isolated AMPAR- and NMDAR-mediated components of the field excitatory postsynaptic potential (fEPSP). The I/O relationships of the AMPAR and NMDAR components were linear across a range of stimulation intensities in both genotypes (Fig. 1 *B* and *C*). Although the slope of the AMPAR I/O curve was comparable in WT and $\beta2m^{-/-}TAP^{-/-}$ slices (Fig. 1*B*), the slope of the I/O curve for NMDAR-mediated responses was significantly steeper in $\beta2m^{-/-}TAP^{-/-}$ slices (Fig. 1*C*; mean NMDAR-mediated I/O slopes: WT 0.21 ± 0.04, $n = 6$ animals; $\beta2m^{-/-}TAP^{-/-}$ 0.41 ± 0.08, $n = 7$ animals; $P < 0.05$). This increase in the NMDAR I/O slope is sufficient to fully account for the drop in the AMPA/NMDA ratio in MHCI-deficient animals and suggests that loss

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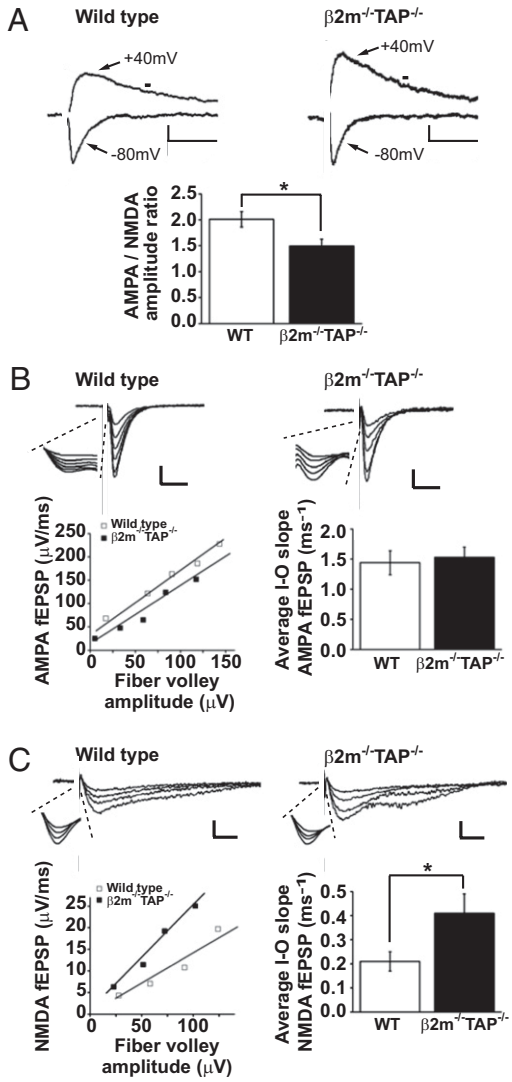

Fig. 1. Increased NMDAR-mediated responses in *β2m*$^{-/-}$*TAP*$^{-/-}$ hippocampal slice. (*A Upper*) Representative EPSCs recorded from individual CA1 pyramidal neurons voltage-clamped at −80 mV or +40 mV. NMDAR-mediated currents were measured at the time marked with horizontal bar. (Scale bar: WT, 20 pA/50 ms; *β2m*$^{-/-}$*TAP*$^{-/-}$, 10 pA/50 ms.) (*Lower*) Mean AMPA/NMDA ratio in CA1 neurons is significantly decreased in *β2m*$^{-/-}$*TAP*$^{-/-}$ animals. (*B Upper*) Representative AMPAR-mediated fEPSPs recorded in D-(−)-2-Amino-5-phosphonopentanoic acid (D-APV) from CA1 dendrites in WT or *β2m*$^{-/-}$*TAP*$^{-/-}$ hippocampal slices. (Scale bar: WT, 0.2 mV/20 ms; *β2m*$^{-/-}$*TAP*$^{-/-}$, 0.1 mV/20 ms.) (*Insets*) Magnified view of the fiber volley. (*Lower Left*) I/O relationship of the AMPAR-mediated responses in the examples above. (*Lower Right*) Summary graph showing mean AMPAR-mediated I/O slopes (WT, *n* = 8 animals; *β2m*$^{-/-}$*TAP*$^{-/-}$, *n* = 8 animals). (*C Upper*) Representative NMDAR-mediated fEPSPs recorded in 6,7-dinitroquinoxaline-2,3-dione (DNQX) from CA1 dendrites in WT or *β2m*$^{-/-}$*TAP*$^{-/-}$ hippocampal slices. (Scale bar: 0.1 mV/20 ms.) (*Insets*) Magnified view of the fiber volley. (*Lower Left*) I/O relationship of the NMDAR-mediated responses in the examples above. (*Lower Right*) Summary graph showing mean NMDAR-mediated I/O slopes (for values, see text).

of MHCI causes a disinhibition of NMDAR-mediated synaptic responses.

Source of Increased NMDAR-Mediated Responses in *β2m*$^{-/-}$*TAP*$^{-/-}$ Hippocampal Neurons. The enhanced NMDAR-mediated responses in *β2m*$^{-/-}$*TAP*$^{-/-}$ neurons might reflect an increase in the proportion of NMDAR-containing, AMPAR-free ("silent") synapses or an increase in the NMDAR-mediated response per synapse. Although silent synapses do not contribute significantly to synaptic transmission at resting membrane potentials, because of blockade of the channel by Mg^{2+}, they could have been unsilenced in the above experiments (by depolarization to +40 mV in the AMPA/NMDA ratio recordings or by lowering extracellular Mg^{2+} in the I/O recordings). To estimate the fraction of silent synapses, we measured the coefficient of variation (CV) of EPSCs evoked by Schaffer collateral stimulation at different holding membrane potentials. The CV of the EPSCs drops when silent synapses are unsilenced and macroscopic currents are comprised of summed activity at a larger number of postsynaptic sites. When the holding potential was switched from −80 mV to +40 mV, the CV dropped to a comparable extent for both WT and *β2m*$^{-/-}$*TAP*$^{-/-}$, suggesting that silent synapses are present in similar proportions regardless of the level of MHCI (Fig. 2*A*). Thus, it is unlikely that the increase in NMDAR-mediated responses in *β2m*$^{-/-}$*TAP*$^{-/-}$ neurons is caused by an increase in the number of silent synapses. Rather, more of the glutamatergic synaptic transmission is mediated by NMDARs at *β2m*$^{-/-}$*TAP*$^{-/-}$ synapses.

An increase in NMDAR-mediated responses could also be caused by changes in NMDAR subunit composition. Most NMDARs are heterotetramers consisting of two obligatory NR1

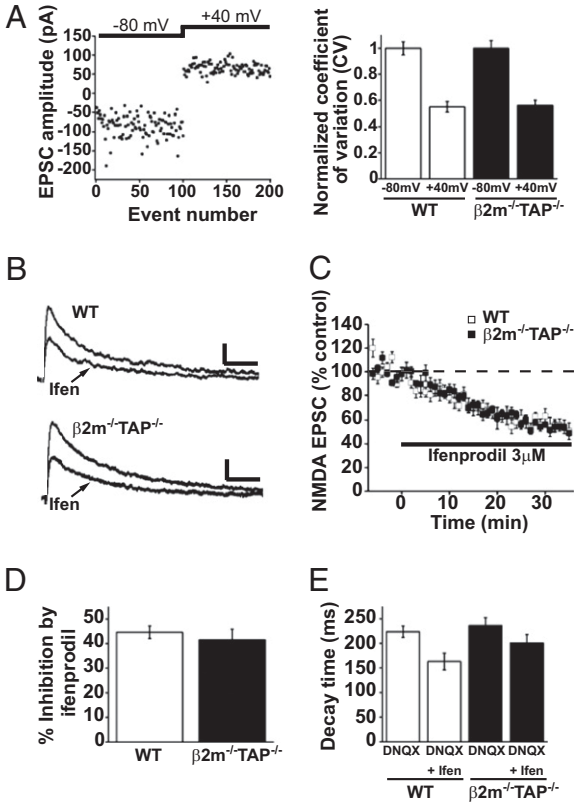

Fig. 2. Normal proportions of silent synapses and NR2B-containing NMDARs in *β2m*$^{-/-}$*TAP*$^{-/-}$ hippocampal neurons. (*A Left*) Sample plot of EPSC amplitudes for individual consecutive events recorded from a WT CA1 neuron voltage-clamped at −80 mV and then shifted to +40 mV. (*Right*) Summary graph showing the mean CV of EPSCs at −80 mV and +40 mV, normalized to the CV at −80 mV (WT *n* = 9 cells; *β2m*$^{-/-}$*TAP*$^{-/-}$ *n* = 8 cells). (*B*) Representative NMDAR-mediated EPSCs recorded from individual WT (*Upper*) or *β2m*$^{-/-}$*TAP*$^{-/-}$ (*Lower*) CA1 neurons 6 min before or 30 min after application of ifenprodil. (Scale bar: 20 pA/100 ms.) (*C*) Averaged NMDAR-mediated EPSCs recorded before and during bath application of 3 μM ifenprodil, normalized to a 6-min baseline (WT *n* = 7 cells; *β2m*$^{-/-}$*TAP*$^{-/-}$ *n* = 8 cells). (*D*) Mean percentage inhibition of the normalized NMDAR-mediated EPSC amplitude by ifenprodil. (*E*) Mean decay time of the NMDAR-mediated EPSC measured 6 min before or 30 min after application of ifenprodil.

subunits paired with two NR2 subunits (NR2A–NR2D). NR2A-containing NMDARs have relatively rapid decay kinetics, whereas NR2B-containing NMDARs, which are more common early in development, have relatively slow decay kinetics (16, 17). An increase in the proportion of NR2B-containing NMDARs can prolong NMDAR activation, enhancing temporal integration and increasing the amplitude of the whole-cell NMDAR-mediated current (18). However, the basal decay kinetics of NMDAR-mediated currents are unchanged in MHCI-deficient neurons (Fig. 2*E*), suggesting that the proportion of NR2B-containing NMDARs may not be altered. To directly determine the contribution of NR2B-containing NMDARs, the component of the EPSC mediated by NR2B-containing NMDARs was blocked with ifenprodil (19, 20). As expected, bath application of ifenprodil caused a reduction of the amplitude and acceleration of the decay of NMDAR-mediated currents that stabilized within 30 min (Fig. 2 *B–E*). Ifenprodil blocked a similar proportion of the NMDAR currents in both genotypes, indicating that NR2B-containing NMDARs make up a normal percentage of the synaptic pool of NMDARs in $\beta2m^{-/-}TAP^{-/-}$ synapses (Fig. 2*D*). Furthermore, endogenous cell-surface NR2B-containing NMDARs were immunolabeled in cultured hippocampal neurons. The intensity of NR2B labeling on the surface of dendrites was indistinguishable in WT and $\beta2m^{-/-}TAP^{-/-}$ neurons (Fig. S2*B*). Thus, three independent measures (EPSC decay kinetics, ifenprodil sensitivity, and NR2B immunostaining) indicate that MHCI does not affect the proportion of NMDAR-mediated currents carried by NR2B-containing receptors. Rather, these results are consistent with the idea that MHCI limits the current carried by both NR2B- and non-NR2B-containing receptors.

Higher levels of the obligatory subunit NR1 at $\beta2m^{-/-}TAP^{-/-}$ synapses could increase the synaptic NMDAR current without affecting the relative contributions of different NR2 subunits. Synaptic levels of NR1 were first evaluated by examining the extent of the colocalization between endogenous NR1 and known synaptic markers in hippocampal neurons in culture. As expected, in mature WT neurons, punctiform NR1 labeling colocalized with PSD-95, a marker of the postsynaptic density, and was directly apposed to SV2, a marker of the presynaptic terminal (Fig. 3 *A* and *B*). In $\beta2m^{-/-}TAP^{-/-}$ neurons, the intensity and degree of colocalization of NR1 with PSD-95 and SV2 was qualitatively similar to levels found for WT neurons (Fig. 3 *A* and *B*). Quantitative analysis confirmed that the average degree of colocalization between NR1 and PSD-95 or SV2 was similar in WT and MHCI-deficient neurons (Fig. 3*C*). To further examine NR1 expression, we performed subcellular fractionation experiments on microdissected hippocampi and compared NR1 levels in Western blots of total (S1) and synaptic (P3) fractions between WT and $\beta2m^{-/-}TAP^{-/-}$ animals. Synaptic fractions were enriched for the synaptically localized protein synaptophysin, demonstrating effective extraction and enrichment of synaptic proteins (Fig. 3*D*). Both total and synaptic levels of NR1 were indistinguishable between WT and $\beta2m^{-/-}TAP^{-/-}$ hippocampal lysates (Fig. 3 *D* and *E*). Additional biochemical experiments showed that the levels of NR1 are also indistinguishable in synaptosomal and PSD fractions from WT versus MHCI-deficient neurons ($\beta2m^{-/-}TAP^{-/-}$ NR1 levels, normalized to WT: synaptosomal fraction, 1.08; PSD fraction, 0.96; $n = 2$). Finally, endogenous cell-surface NR1 was immunolabeled in cultured hippocampal neurons. Characteristic, punctuate NR1 staining was observed on the surface of dendrites in WT and $\beta2m^{-/-}TAP^{-/-}$ neurons. Quantitative analysis showed no increase but a modest yet significant decrease in the intensity of NR1 labeling in proximal dendrites in $\beta2m^{-/-}TAP^{-/-}$ neurons (Fig. S2). Because NR1 is an obligatory subunit of all NMDARs, the relative stability of NR1 levels and localization in both immunocytochemical and biochemical assays is not consistent with an increase in the number of functional NMDARs in $\beta2m^{-/-}TAP^{-/-}$ hippocampal neurons.

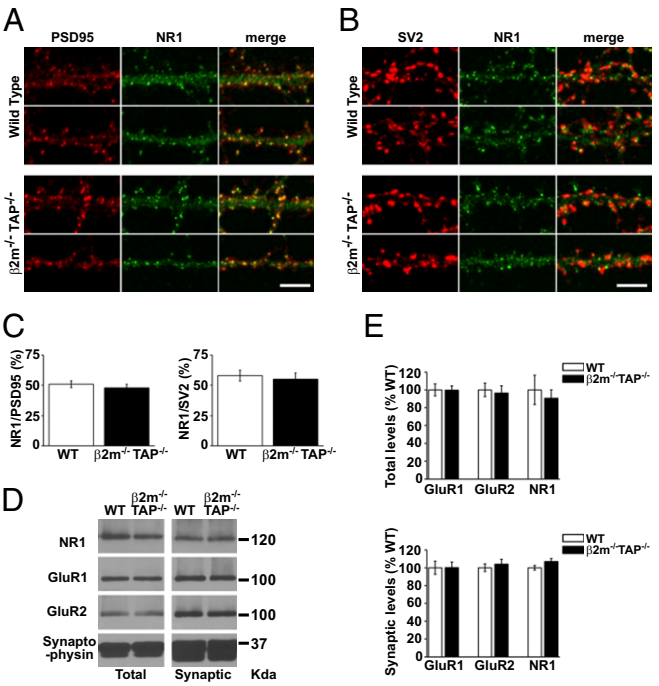

Fig. 3. Total and synaptic levels of NR1, GluR1, and GluR2 are not increased in $\beta2m^{-/-}TAP^{-/-}$ hippocampal neurons. (*A*) Representative NR1 and PSD-95 double-label immunostaining in straightened proximal dendrites from WT and $\beta2m^{-/-}TAP^{-/-}$ hippocampal neurons in culture. (Scale bar: 5 μm.) (*B*) Representative NR1 and SV2 double-label immunostaining in straightened proximal dendrites from WT and $\beta2m^{-/-}TAP^{-/-}$hippocampal neurons in culture. (Scale bar: 5 μm.) (*C*) Mean percentage of NR1 puncta colocalizing with PSD-95 puncta *(Left*; WT $n = 12$ cells; $\beta2m^{-/-}TAP^{-/-}$ $n = 13$ cells) or SV2 puncta (*Right*; WT $n = 11$ cells; $\beta2m^{-/-}TAP^{-/-}$ $n = 12$ cells) in WT versus $\beta2m^{-/-}TAP^{-/-}$ hippocampal neurons in culture (two separate experiments). Colocalization was defined as contact between puncta at the light level, and therefore includes closely apposed as well as extensively overlapping puncta. (*D*) Representative Western blot of total (S1) and synaptic plasma membrane–enriched (P3) fractions from WT and $\beta2m^{-/-}TAP^{-/-}$ mouse hippocampi probed for NR1, GluR1, GluR2, and synaptophysin. (*E*) Total (*Upper*) or synaptic (*Lower*) levels of GluR1, GluR2, and NR1 in samples from four WT and four $\beta2m^{-/-}TAP^{-/-}$ animals, normalized to synaptophysin and represented as percentage of WT.

Changes in the synaptic contribution of NR3 subunits (21) could also potentially account for the increase in NMDAR-mediated current observed in $\beta2m^{-/-}TAP^{-/-}$ neurons. However, total and synaptic levels of NR3A were similar between WT and $\beta2m^{-/-}TAP^{-/-}$ hippocampal lysates (Fig. S3). Together, our results suggest that MHCI limits the function of NMDARs without affecting receptor levels, subunit composition, or gross subcellular localization.

NMDA-Induced Changes in AMPAR Trafficking. Activation of NMDARs gives rise to characteristic patterns of AMPAR trafficking during the expression of NMDAR-dependent plasticity. For example, brief bath application of NMDA causes a long-lasting NMDAR-dependent synaptic depression in WT hippocampus that is associated with removal of AMPARs from the cell surface (22–24). Therefore, to assess the impact of MHCI on NMDAR-induced AMPAR trafficking, we measured cell-surface levels of the AMPAR subunits GluR1 and GluR2 before and after direct stimulation of NMDARs. Subcellular fractionation experiments showed that basal total and synaptic levels of GluR1 and GluR2 were indistinguishable from WT in $\beta2m^{-/-}TAP^{-/-}$ hippocampal lysates (Fig. 3 *D* and *E*). Similarly, characteristic, punctuate cell-surface GluR1 and GluR2 labeling was observed on dendrites of both WT and $\beta2m^{-/-}TAP^{-/-}$ cultured hippocampal neurons before NMDA treatment (Fig. 4 *A* and *C*), and

quantitative analysis confirmed that the average basal intensity of cell-surface GluR1 and GluR2 immunolabeling was similar in WT and MHCI-deficient neurons (Fig. 4 *B* and *D*). Twelve minutes after NMDA application, WT neurons showed on average no significant change in cell-surface GluR1 or GluR2 levels (Fig. 4), consistent with previous results at this time point (23–25). In contrast, NMDA treatment induced a rapid and significant *increase* in cell-surface GluR1 in $\beta2m^{-/-}TAP^{-/-}$ neurons [WT $7.25 \pm 1.8\%, n = 43$ cells; $\beta2m^{-/-}TAP^{-/-}$ $19.2 \pm 3.4\%, n = 47$ cells; $^*P < 0.001$, Kruskal–Wallis test (nonparametric ANOVA followed by Dunn posttest)] (Fig. 4 *A* and *B*). NMDA also increased cell-surface GluR2 levels in these transgenic animals, although this trend was not statistically significant (Fig. 4 *C* and *D*). Surface biotinylation experiments performed on hippocampal neurons in culture showed similar findings (Fig. S4). Together, these results suggest that MHCI levels determine NMDAR-induced AMPAR trafficking events during the expression of plasticity.

Discussion

The present studies show that, in addition to its immune role, MHCI is an essential modulator of NMDAR function and AMPAR trafficking in the mammalian CNS. At MHCI-deficient synapses, NMDAR-mediated responses are enhanced, even in the absence of significant changes in NMDAR levels, distribution, or subunit composition. Basal expression, trafficking, and function of AMPARs are normal in these neurons, but NMDAR-induced AMPAR trafficking is altered. Thus, endogenous MHCI normally limits NMDAR currents and is required for appropriate NMDAR-induced AMPAR trafficking, a central event in the expression of NMDAR-dependent synaptic plasticity.

A key prediction of the current results is that, by limiting NMDAR-mediated responses, MHCI regulates NMDAR-dependent synaptic plasticity in the hippocampus. In prevailing models, the level and kinetics of NMDAR activation determine the sign and magnitude of plasticity (14, 15). The current data support this model and suggest that enhanced NMDAR activation may underlie the enhancement of LTP and loss of LTD in the hippocampus of MHCI-deficient animals (4). Such shifts in plasticity are critical to brain development and memory storage. Our data indicate that MHCI could potentially regulate the balance between LTP and LTD at central synapses by tuning NMDAR-mediated responses. MHCI is required for scaling of miniature EPSC amplitude and PSD-95 puncta size in response to chronic activity blockade in vitro (9), demonstrating that, in addition to its effects on acute synaptic plasticity, MHCI can also regulate homeostatic plasticity. Whether the effects of MHCI on homeostatic and acute forms of plasticity are both mediated by regulation of NMDAR function remains to be determined.

Here we identify changes in NMDAR-induced AMPAR trafficking as a likely cellular source of altered synaptic plasticity in MHCI-deficient animals. In $\beta2m^{-/-}TAP^{-/-}$ neurons, NMDA treatment causes an aberrant increase in cell-surface GluR1. Although the origin of these receptors is unknown, the increase in cell-surface GluR1 is rapid, consistent with a relocalization of preexisting AMPARs rather than de novo synthesis of AMPARs. A similar rapid insertion of AMPARs is seen during chemically induced LTP, when cell-surface GluR1 levels rise because of delivery of GluR1 from recycling pools (26, 27). Thus, by modifying NMDAR function, MHCI may change the coupling between a given plasticity-inducing stimulus and downstream AMPAR trafficking events.

NMDAR function can be regulated at many levels, including changes in receptor expression, subcellular localization, subunit composition, trafficking, phosphorylation, and interactions with cofactors and scaffolding molecules (17, 28). Although the NMDAR-mediated response is increased at MHCI-deficient synapses, we did not detect an increase in the total, surface, or synaptic levels of the obligatory NMDAR subunit NR1 or any

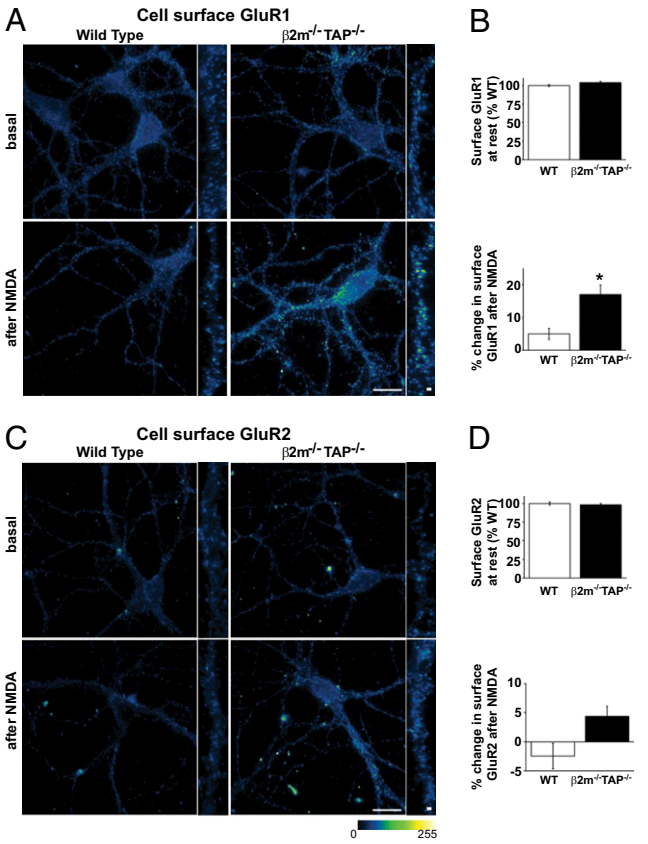

Fig. 4. NMDA increases cell-surface GluR1 levels in $\beta2m^{-/-}TAP^{-/-}$ neurons. (*A*) Representative pseudocolored cell-surface GluR1 immunostaining in WT and $\beta2m^{-/-}TAP^{-/-}$ hippocampal neurons in culture at rest (basal) or 12 min after NMDA treatment. (Scale bar: 20 μm; high magnification: 1 μm.) (*B*) Quantification of dendritic cell-surface GluR1 labeling (four separate experiments). (*Upper*) Pooled data at rest. (*Lower*) Significant increase in cell-surface GluR1 labeling after NMDA treatment in $\beta2m^{-/-}TAP^{-/-}$ animals. (*C*) Representative pseudocolored cell-surface GluR2 immunostaining in WT and $\beta2m^{-/-}TAP^{-/-}$ hippocampal neurons in culture at rest (basal) or 12 min after NMDA treatment. (Scale bar: 20 μm; high magnification: 1 μm.) (*D*) Quantification of dendritic cell-surface GluR2 labeling (four separate experiments). (*Upper*) Pooled data at rest. (*Lower*) Change in surface GluR2 after NMDA treatment (WT $n = 42$ cells; $\beta2m^{-/-}TAP^{-/-}$ $n = 38$ cells).

change in the number of silent synapses. Furthermore, both electrophysiological and immunocytochemical data indicate that the proportion of NR2B-containing receptors is normal. Biochemical results indicate that there is also no increase in the levels of NR3A. Together, these results indicate that MHCI does not regulate NMDAR levels and does not cause gross changes in receptor trafficking or proportions of NR2B- or NR3A-containing receptors. How then does MHCI limit the function of synaptic NMDARs?

One possibility is that MHCI affects the composition of NR2B- and NR3A-free NMDARs. NR2C and NR2D are expressed in mammalian brain, and, although NR2C is not expressed in the hippocampus, NR2D is detected in the hippocampus at the ages we examined (17). However, NR2D subunits confer significantly slower decay kinetics to NMDAR-mediated currents (29). The decay kinetics of whole-cell NMDAR-mediated currents are normal in MHCI-deficient animals (Fig. 2*E*), and therefore it is unlikely that an increase in the contribution of NR2D underlies the increase in NMDAR-mediated responses. It is also unlikely that changes in the probability of release contribute to the increase in NMDAR-mediated responses that we observed because an increase in presynaptic glutamate release should also enhance AMPAR-mediated currents at the same synapses (e.g., ref. 30).

A second possibility is that MHCI affects the population of NMDARs that are localized to synaptic versus immediately perisynaptic compartments. Synaptic levels of NR1 are not altered in biochemical fractionation experiments, but this method enriches synaptic as well as immediately perisynaptic receptors, the latter of which are not thought to contribute to basal synaptic transmission. Thus, a relocalization of NMDARs from perisynaptic to synaptic sites in $\beta2m^{-/-}TAP^{-/-}$ neurons could yield an increase in NMDAR-mediated responses without an apparent change in NMDAR synaptic levels, when measured biochemically. However, more spatially precise immunostaining experiments show that the colocalization of NR1 with markers of synaptic sites (SV2 and PSD-95) is unchanged in $\beta2m^{-/-}TAP^{-/-}$ neurons, suggesting it is unlikely that changes in NR1 localization contribute significantly to the changes in NMDAR-mediated responses. In the future, immunoelectron microscopy could be used to determine whether MHCI affects the perisynaptic levels of NMDARs.

A third possibility is that MHCI mediates posttranslational changes in the functional properties of synaptic NMDARs. NMDARs are regulated posttranslationally by phosphorylation as well as by interactions with soluble cofactors [e.g., glycine, D-serine, Mg^{2+}, protons, zinc, polyamines, and dynorphin (17)] and transmembrane proteins [e.g., dopamine receptors, EphB receptors, and metabotropic glutamate receptors (31–33)]. Regardless of whether MHCI limits NMDARs directly or indirectly, the relevant modification may lie in the obligatory NR1 subunit because MHCI has similar effects on both NR2B- and non-NR2B-containing NMDARs.

MHCI is expressed in dendrites of hippocampal neurons, where it colocalizes with the postsynaptic marker PSD-95 (9), suggesting that MHCI could regulate dendritic NMDAR-mediated responses in a cell-autonomous manner (i.e., in *cis*). However, a recent study using immunogold electron microscopy found that MHCI is detectable at both pre- and postsynaptic sites in rat visual cortex (34). Until similar studies are conducted in the hippocampus, we cannot exclude the possibility that MHCI may also be expressed in presynaptic terminals in the hippocampus and may affect NMDAR-mediated responses in *trans*. Moreover, there is increasing evidence supporting a role for glial cells in modulating synaptic transmission, and NMDAR-mediated currents in particular (35, 36). In addition to being expressed by neurons, low levels of MHCI molecules are present on astrocytes and microglia in healthy brains (37, 38). Therefore, it is possible that MHCI regulates NMDARs through neuron–glia interactions.

A number of immunoreceptors that can bind to MHCI are expressed in the hippocampus, including PirB, Ly49, and KIR-like receptors (39–41). It is unlikely that MHCI affects NMDAR-dependent synaptic transmission via PirB, however, because recent studies show that LTP and LTD are normal in PirB knockouts (42). Further studies will be necessary to evaluate the role played by other immunoreceptors in MHCI functions at hippocampal synapses. Outside the CNS, MHCI is known to bind to cell-surface proteins both in *cis* and in *trans* (43). Studies consistent with a *trans* effect of MHCI on presynaptic ultrastructure and synapsin expression, as well as a *cis* effect on the scaling of the size of PSD-95 puncta in response to activity blockade, have recently been published (9). Thus, MHCI may have neuronal effects both in *cis* and in *trans*, depending on the brain region and function. Our current results suggest that the dominant changes in both basal synaptic transmission and plasticity in MHCI-deficient CA3–CA1 synapses occur postsynaptically. Thus, the most parsimonious explanation is that postsynaptically expressed MHCI affects postsynaptic NMDAR-mediated responses in a cell-autonomous manner. Similar modulation of NMDAR function in *cis* has been demonstrated for other transmembrane proteins, including dopamine receptors and EphB receptors (31, 33).

Given the central importance of NMDARs in the control of gene expression, brain development, synaptic plasticity, learning and memory, and excitotoxicity as well as accumulating evidence of glutamatergic dysfunction in neurological disorders, including autism and schizophrenia (28), it is essential to understand mechanisms that control NMDAR efficacy. Here we provide evidence that endogenous MHCI limits NMDAR currents in the mammalian CNS. MHCI levels are dynamic during development and are regulated by activity (3), and thus our results suggest a mechanism whereby changes in MHCI levels could link developmental stage and synaptic activity to physiological changes in the rules governing synaptic plasticity in vivo. Neuronal MHCI levels are also increased during inflammation (44), seizures (3), injury (45), and aging (46). By limiting NMDAR function, higher levels of MHCI under these conditions could prevent runaway potentiation and act as an endogenous neuroprotective against NMDAR-mediated excitotoxicity.

Materials and Methods

Mice. Experiments were performed on commercially available C57BL/6 mice and MHCI-deficient mice in a C57BL/6 background, backcrossed more than nine times to WT. Because many MHCI genes are expressed in neurons, we made use of mice genetically deficient for two molecules required for the stable cell-surface expression of nearly all MHCI proteins: β2-microglobulin (β2m), an obligatory MHCI subunit, and the transporter associated with antigen processing (TAP1), a transporter required to load peptides onto mature MHCI proteins (47–49). Animals lacking these two proteins ($\beta2m^{-/-}TAP^{-/-}$ double mutants) are immune-compromised but are outwardly normal when kept in a clean facility. All mice were age- and sex-matched within experiments, and procedures were performed according to institutional guidelines and protocols approved by the University of California at San Diego Institutional Animal Care and Use Committee.

Electrophysiology. Acute coronal brain slices (350 μm) were prepared from postnatal day 13 (P13) to P16 C57/Bl6 WT or $\beta2m^{-/-}TAP^{-/-}$ mice. Visualized whole-cell patch-clamp recordings of evoked EPSCs from individual CA1 pyramidal neurons and field recordings from populations of CA1 pyramidal cells were conducted at room temperature (~25 °C) with standard methods. See *SI Materials and Methods* for details.

Hippocampal Cultures. Low-density cultures of acutely dissociated hippocampal neurons were prepared from newborn (P0) WT and $\beta2m^{-/-}TAP^{-/-}$ mice by using a protocol adapted from ref. 50. See *SI Materials and Methods* for details.

Glutamate Receptor Immunocytochemistry. ***Surface labeling.*** Endogenous AMPARs and NMDARs were labeled in hippocampal neurons in culture with antibodies directed against the extracellular domain of GluR1 (rabbit anti-GluR1; Calbiochem), GluR2 (mouse anti-GluR2 clone 6C4; Zymed), NR1 (mouse anti-NR1 clone 54.1; BD Pharmingen), or NR2B (mouse anti-NR2B clone N59/20; NeuroMab).
Double-label immunostaining. Hippocampal neurons were double-labeled with anti-NR1 (rabbit anti-NR1; Millipore) and anti–PSD-95 (mouse anti–PSD-95 clone K28/43; NeuroMab) or anti-SV2 (mouse anti-SV2 clone SP2/0; Developmental Studies Hybridoma Bank). See *SI Materials and Methods* for details.

Image Acquisition and Quantification. ***Surface labeling.*** Images were acquired by using an epifluorescence microscope (Olympus BX51WI) equipped with a CCD camera (Qimaging Retiga 2000R). For comparisons between genotypes, all images were acquired the same day using identical acquisition settings. Images were quantified by an observer blind to genotype with ImageJ software (National Institutes of Health, version 1.37). See *SI Materials and Methods* for details.
Double-label immunostaining. Images were acquired by using an inverted microscope (Leica DMI6000) outfitted with a spinning disk confocal head (Yokogawa) and equipped with a cooled CCD camera (Hamamatsu). For comparisons between genotypes, all images were acquired the same day using identical acquisition settings. Maximum projected confocal *Z*-stacks are displayed.

Subcellular Fractionation. For each experiment, two 4- to 5-wk-old animals of each genotype were used. Subcellular fractionation was performed as previously described (51). See *SI Materials and Methods* for details.

Western Blot Analysis. Protein quantification was performed by using a BCA protein assay kit (Pierce) according to the manufacturer's instructions. Thirty

micrograms of each sample was subjected to SDS/PAGE, transferred to a PVDF membrane, and probed with antibodies directed against proteins of interest: GluR1 (rabbit anti-GluR1, 0.1 μg/mL; Chemicon), GluR2 (mouse anti-GluR2 clone 6C4, 0.5 μg/mL; Zymed), NR1 (mouse anti-NR1 clone 54.1, 0.5 μg/mL; BD Pharmingen), NR3A (rabbit anti-NR3A, 1 μg/mL; Chemicon), and synaptophysin (clone SY38, 0.3 μg/mL; Chemicon). Relative band intensity was quantified by densitometric analysis with ImageJ software (National Institutes of Health, version 1.37). Sample bands were normalized to synaptophysin and averaged across experiments.

Statistics. For all experiments, means are reported ± SEM. Statistical comparisons of the data were performed with GraphPad InStat version 3.06 for Windows (GraphPad Software).

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