The high sequence diversity in *H. pylori* allows the recognition of distinct populations after centuries of coexistence in individual geographic locations, as demonstrated in the Americas and South Africa. Even after thousands of years of contact in Europe between bacteria introduced by distinct waves of migration, residual short-range linkage disequilibrium has allowed us to identify ancestral chunks of chromosome. Thus, analysis of *H. pylori* from human populations could also help resolve details of human migrations.

Elucidation of the pattern of population subdivision is also of medical relevance (*25*). Geographically variable results regarding the association of putative virulence factors with disease (*26*) might well reflect differences in the local prevalence of the individual *H. pylori* populations. Similarly, the development of diagnostic tests, antibiotics, and vaccines needs to account for global diversity and will be aided by the availability of representative isolates.

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Experience Strengthening Transmission by Driving AMPA Receptors into Synapses

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The mechanisms underlying experience-dependent plasticity in the brain may depend on the AMPA subclass of glutamate receptors (AMPA-Rs). We examined the trafficking of AMPA-Rs into synapses in the developing rat barrel cortex. In vivo gene delivery was combined with in vitro recordings to show that experience drives recombinant GluR1, an AMPA-R subunit, into synapses formed between layer 4 and layer 2/3 neurons. Moreover, expression of the GluR1 cytoplasmic tail, a construct that inhibits synaptic delivery of endogenous AMPA-Rs during long-term potentiation, blocked experience-driven synaptic potentiation. In general, synaptic incorporation of AMPA-Rs in vivo conforms to rules identified in vitro and contributes to plasticity driven by natural stimuli in the mammalian brain.

The modifications that occur in the vertebrate brain as a consequence of experience are poorly understood, although changes at excitatory synapses may encode learning (*1–4*). Fast excitatory transmission in the central nervous system of vertebrates is largely mediated by the actions of glutamate on AMPA-Rs (*5*). The number of AMPA-Rs at a synapse can control the coupling strength between pre- and postsynaptic neurons, and thus serves as a key control site for neural function (*6–10*).

AMPA-Rs are multimeric complexes composed of subunits GluR1 to GluR4 (*11*). In vitro, subunit-specific rules govern the incorporation of AMPA-Rs into synapses (*12–14*). For example, plasticity-inducing protocols and *N*-methyl-D-aspartate receptor (NMDA-R) activation are required before AMPA-Rs with subunits containing long cytoplasmic tails (such as GluR1) can be driven into synapses, thus enhancing transmission. In contrast, AMPA-Rs comprising only subunits containing short cytoplasmic tails (GluR2 and GluR3) continuously replace synaptic receptors in a manner that maintains transmission (fig. S1C).

To examine the effects of experience on AMPA-R trafficking in vivo, we delivered AMPA-R subunits to a small number of neurons (~100) in rat barrel cortex by in vivo microinjection of an expressing Sindbis virus (Fig. 1A) (fig. S1, A and B) (*15–17*) at postnatal day (PND) 12, an age characterized by rapid experience-dependent development of barrel cortex circuitry (*18, 19*). Sensory experience was controlled by either preserving or trimming whiskers (*19–21*), and 2 days later, coronal brain slices were prepared (fig. S1B).

To determine whether AMPA-Rs are driven into synapses by experience, we examined brain slices from animals infected with a virus producing GluR1 (*22*). Infected neurons in layer 2/3 were identified by the green fluorescent protein (GFP) tag on GluR1 (fig. S1B). Whole-cell recordings were obtained from nearby infected and uninfected neurons, allowing direct comparison of synaptic responses evoked with stimulating electrodes placed in layer 4. AMPA-R–mediated responses were isolated pharmacologically (*22*). Transmission mediated by activation of AMPA-Rs on neurons expressing GluR1-GFP showed significantly increased rectification (ratio of response at −60 mV to response at +40 mV) compared to transmission onto nearby control neurons (Fig. 1). Neurons expressing only GFP in animals exposed to the same experimental protocol showed no change in rectification (Fig. 1D). The increased rectification is a signature of recombinant homomeric GluR1 receptors and indicates their delivery into synapses (*12, 23, 24*).

In vitro, GluR1-GFP cannot be driven into synapses unless long-term potentiation (LTP)

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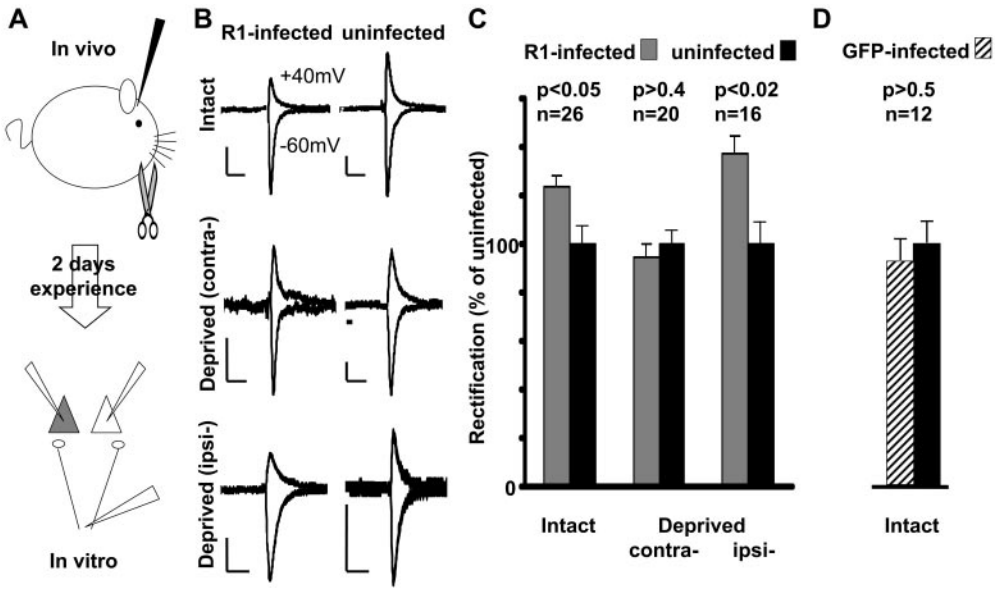

Fig. 1. Experience drives recombinant homomeric GluR1 into synapses. (**A**) Experimental protocol [see text and (*22*) for details]. Triangles represent nearby infected (gray) and uninfected (white) neurons in layer 2/3 cortical slices recorded while stimulating layer 4. (**B**) Synaptic responses (average of 50 consecutive trials) recorded from layer 2/3 cortical slice neurons (held at −60 mV and +40 mV, as indicated) infected or uninfected with virus expressing GluR1-GFP. Cortical slices were obtained from animals treated as indicated. Note increased rectification in infected neurons only in regions contralateral to spared whiskers and its abolishment by deprivation. Scale bars, 10 pA (vertical), 20 ms (horizontal). (**C**) Graph of average rectification index (RI; response at −60 mV / response at +40 mV) of synaptic responses from layer 2/3 pyramidal neurons expressing GluR1-GFP (gray) or uninfected (black), normalized to RI value of nearby uninfected cells. Experimental condition is indicated below. Number of recordings and statistical difference (Student's *t* test) are indicated. Neurons expressing GluR1-GFP showed no increase in amplitude at −60 mV (infected, 20.9 ± 5 pA; uninfected, 18.9 ± 3 pA; $N = 14$, $P > 0.5$, Wilcoxon; see fig. S3). (**D**) Neurons expressing only GFP showed no change in RI values as compared to uninfected cells.

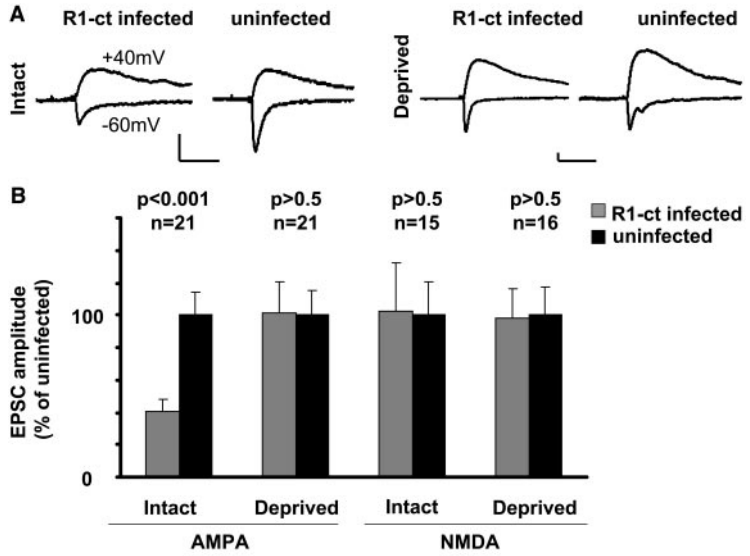

Fig. 2. Whisker-mediated experience drives endogenous GluR1-containing receptors to synapses. (**A**) Whole-cell recordings of transmission between layer 4 and layer 2/3 pyramidal neurons simultaneously recorded from postsynaptic neurons infected (left) and uninfected (right) with virus expressing GluR1 cytoplasmic tail (GluR1-ct-GFP). Left traces, whiskers intact; right traces, whiskers trimmed from PND 12 to PND 14 contralateral to recording region. Scale bars, 10 pA, 40 ms. (**B**) Graph of mean AMPA-R– and NMDA-R–mediated transmission onto infected (gray) and uninfected (black) neurons simultaneously recorded from layer 2/3, normalized to values obtained in uninfected neurons. Number of recordings and statistical analysis (Wilcoxon nonparametric test) are shown.

is first induced; spontaneous neural activity is not sufficient (*23*). Is experience required for incorporation of recombinant GluR1 receptors into synapses? We examined brain slices from animals that expressed GluR1-GFP between PND 12 and PND 14 with whiskers trimmed on one side of the face. Brain regions contralateral to trimming [thus lacking somatosensory input (*25*)] showed no increased rectification, indicating that recombinant receptors were not incorporated into synapses, whereas neurons ipsilateral to trimmed whiskers showed increased rectification (Fig. 1). These results indicate that experience during PND 12 to PND 14 drives recombinant homomeric GluR1-GFP receptors into synapses between layer 4 and layer 2/3 neurons.

To examine whether endogenous GluR1-containing receptors are driven into synapses by experience, we used a virus expressing the cytoplasmic tail of GluR1 tagged with GFP (GluR1-ct-GFP). This construct can bind to proteins normally associated with GluR1 and thereby prevent delivery of GluR1-containing receptors to synapses during LTP (*12*). We reasoned that if experience enhances transmission by driving endogenous GluR1-containing AMPA-Rs into synapses, this enhanced transmission should be blocked, and neurons expressing GluR1-ct-GFP should show weaker transmission than uninfected neurons. We examined transmission in brain slices from animals with intact whiskers expressing GluR1-ct-GFP between PND 12 and PND 14. Transmission onto uninfected neurons was greater than transmission onto neurons expressing GluR1-ct-GFP by a factor of ~2.5 (Fig. 2) (*26*). Neurons expressing only GFP showed no decrease in transmission (infected, 23.7 ± 4 pA; uninfected, 24.7 ± 4 pA; $N = 19$, $P > 0.5$). This finding supports the view that synaptic delivery of endogenous GluR1-containing receptors is blocked by GluR1-ct-GFP. The effect of GluR1-ct-GFP was specific for AMPA-R–mediated transmission, as NMDA-R–mediated transmission was not affected (Fig. 2).

To test whether GluR1-ct-GFP expression blocked experience-driven plasticity, we examined the effects of this construct in deprived animals. We reasoned that if synaptic delivery of GluR1 receptors does not occur during sensory deprivation, then GluR1-ct-GFP should show no effect on transmission. As predicted, transmission onto neurons expressing GluR1-ct-GFP was no different

from transmission onto nearby uninfected neurons in slices prepared from animals deprived during GluR1-ct-GFP expression (Fig. 2). The effect of GluR1-ct-GFP expression on transmission in nondeprived animals was significantly different from that in deprived animals, as determined by calculating a depression index [(infected – uninfected)/(infected + uninfected)], which was significantly lower in nondeprived (-0.41 ± 0.08) than in deprived (0.0 ± 0.1, $P < 0.02$) animals.

These findings support the view that sensory experience drives synaptic delivery of endogenous GluR1-containing receptors. This view is further supported by another measure of endogenous AMPA receptor synaptic delivery, the ratio of AMPA to NMDA responses (A/N). In brain slices from animals deprived between PND 12 and PND 14, the A/N for transmission between layer 4 and layer 2/3 was significantly lower than that observed in nondeprived animals of the same age (A/N intact $= 2.9 \pm 0.4$, $n = 15$; A/N deprived $= 1.4 \pm 0.3$, $n = 11$; $P < 0.02$).

In vitro, activity-driven delivery of AMPA-Rs containing long cytoplasmic tails into synapses enhances transmission. While potentiation persists over days, the presence of receptors with long cytoplasmic tails at synapses is transient, as they are replaced by receptors containing only short cytoplasmic tails [such as GluR2 (*24*)]. To test whether experience-driven synaptic delivery of AMPA-Rs follows a similar trajectory, we infected animals with a virus encoding GluR1-GFP at PND 12 and allowed 2 days of experience and one subsequent day of deprivation before cortical slices were prepared. Transmission between layer 4 and infected layer 2/3 neurons showed no increase in rectification (Fig. 3). Three days of GluR1-GFP expression with no deprivation led to the expected rectification (Fig. 3). These results are consistent with in vitro experiments (*24*), suggesting that channels with long cytoplasmic tails driven to synapses by activity exist only transiently at synapses.

On the basis of in vitro studies (*6–9, 12, 27*), we predict that replacement of synaptic AMPA-Rs by GluR2-containing receptors does not require experience. Animals were infected at PND 12 with GluR2(R586Q)-GFP, a GluR2 mutant enabling electrophysiological tagging (*12*), and cortical slices were prepared at PND 14. These receptors were incorporated into synapses regardless of deprivation (Fig. 4). To examine endogenous GluR2-dependent trafficking to synapses, we infected animals with GluR2-ct-GFP, a construct that can block the continual cycling of AMPA-Rs containing GluR2 and GluR3 (*12*). In slices prepared from animals with whiskers intact or trimmed, transmission was depressed in infected layer 2/3 neurons relative to near-

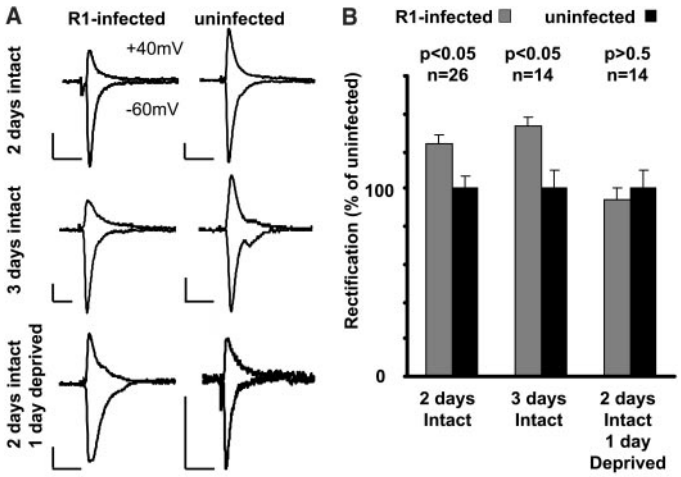

Fig. 3. Synaptically delivered recombinant GluR1 receptors are replaced by endogenous GluR2-containing receptors. (**A**) Animals were infected with Sindbis virus carrying recombinant GluR1-GFP on PND 12, and returned to home cage for 2 or 3 days with intact whiskers; one group of animals had whiskers trimmed at day 2 contralateral to site of injection. Sample traces were obtained from infected and uninfected cells of animals as indicated. Scale bars, 10 pA, 20 ms. (**B**) RI values of GluR1-GFP–expressing and uninfected layer 2/3 pyramidal neurons, normalized to RI values of uninfected cells. Data for 2 days intact group are the same as shown in Fig. 1. Number of recordings and statistical significance (Student's *t* test) are indicated.

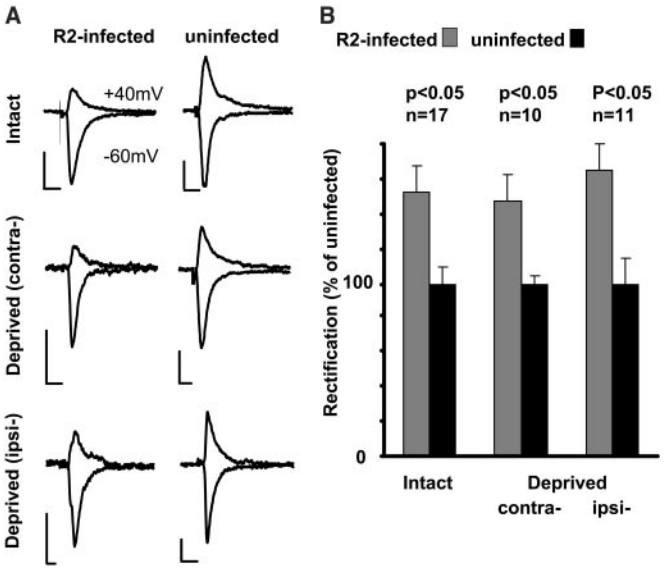

Fig. 4. Synaptic delivery of recombinant GluR2-containing receptors is not blocked by whisker deprivation. Same experiment as Fig. 1, except neurons were infected with virus producing GluR2(R586Q)-GFP. (**A**) Sample traces of transmission obtained from cortical slices of animals as indicated. Scale bars, 10 pA, 10 ms. (**B**) Same as Fig. 1C, for neurons expressing GluR2(R586Q)-GFP.

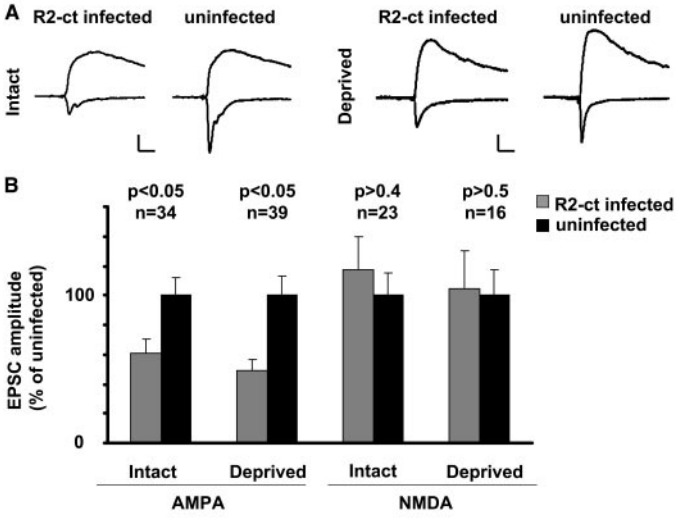

Fig. 5. Synaptic delivery of endogenous GluR2-containing receptors is not blocked by whisker deprivation. Same experiment as Fig. 2, except neurons were infected with virus producing the carboxyl tail of GluR2 (GluR2-ct-GFP). (**A**) Sample traces of transmission obtained from cortical slices of animals as indicated. Scale bars, 20 pA, 20 ms. (**B**) Same as Fig. 2B, for neurons expressing GluR2-ct-GFP.

by control cells (Fig. 5). These results indicate that GluR2-dependent AMPA-R cycling in and out of synapses previously described in vitro (*6–9, 12, 27*) occurs in vivo and does not require experience (*22*).

Considerable progress has been made in uncovering the cellular and molecular mechanisms underlying activity-dependent synaptic plasticity in vitro. Although LTP is a leading contender as a mechanism to encode experience in brain circuits, few reports (*20, 28, 29*) suggest that LTP occurs in vivo in response to natural stimuli. Here, we tested whether synaptic modifications identified to occur during LTP in vitro are also driven by experience in the intact brain. We examined excitatory transmission between layer 4 and layer 2/3 neurons in barrel cortex during a period when considerable experience-dependent plasticity occurs (*18, 19*). For instance, between PND 12 and PND 14 there is a doubling of the number of synapses in barrel cortex (*18*). Synapse numbers appear unaffected by sensory deprivation (*30, 31*); nonetheless, other aspects of synaptic function, such as receptor content, could be dependent on experience.

In agreement with in vitro models indicating activity-dependent synaptic incorporation (*12, 13*), we find that recombinant GluR1 is driven into synapses by experience. Furthermore, GluR1-ct, which can block LTP in vitro (*23*), prevents experience-driven synaptic potentiation. In accordance with in vitro studies (*6–9, 12, 27*), we find that replacement of synaptic receptors depends on interactions by the GluR2 cytoplasmic tail and that it can occur in the absence of plasticity-inducing experience. Thus, the presence of AMPA-Rs with long cytoplasmic tails at a synapse may represent the signature of recent experience-driven plasticity. This study reinforces the view that concepts and reagents derived from in vitro LTP studies can provide strategies to elucidate experience-driven synaptic plasticity in specific brain regions.

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