The effects of our amendments on both the POA/SOA split and the total OA burden are shown in Fig. 4. The traditional model predicts substantial contributions from POA, whereas the revised model predicts that ambient OA is dominated by SOA during the summer. Such a shift is consistent with recent field measurements indicating dominant contributions from SOA (8–10) while remaining consistent with POA estimates based on low-volatility tracers. In terms of the overall OA budget, the revised model decreases predicted OA in urban areas by as much as 50% and increases it in many rural areas by 15 to 30% (Fig. 4C), reducing the large urban-to-regional gradients predicted by the traditional model and resulting in considerably better agreement with measured urban-to-regional OA ratios (Fig. 4D).

This work has several implications for our understanding of OA. The semivolatile character of primary emissions requires that instead of measuring fixed POA EFs, we must measure the volatility distribution of the emissions. Models and inventories must account for these distributions and their evolution with photochemical age. Regulations and control technologies may also need to be revised to control SVOC and IVOC emissions because of their importance as SOA precursors. The results also imply that, except for people living close to sources, the majority of the population (even in urban areas) is exposed mostly to SOA. Ultimately, a relatively local urban emissions problem is transformed into a regional source of oxidized and presumably hydrophilic OA. The health consequences and climate effects of this oxidized material are almost certainly dramatically different from those of primary emissions.

References and Notes

Anti-Hebbian Long-Term Potentiation in the Hippocampal Feedback Inhibitory Circuit
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Long-term potentiation (LTP), which approximates Hebb’s postulate of associative learning, typically requires depolarization-dependent glutamate receptors of the NMDA (N-methyl-d-aspartate) subtype. However, in some neurons, LTP depends instead on calcium-permeable AMPA-type receptors. This is paradoxical because intracellular polyamines block such receptors during depolarization. We report that LTP at synapses on hippocampal interneurons mediating feedback inhibition is “anti-Hebbian”: It is induced by presynaptic activity but prevented by postsynaptic depolarization. Anti-Hebbian LTP may occur in interneurons that are silent during periods of intense pyramidal cell firing, such as sharp waves, and lead to their altered activation during theta activity.

Associative N-methyl-d-aspartate receptor (NMDAR)-dependent LTP is induced by coincident activity in afferent pathways sufficient to depolarize postsynaptic neurons (1). However, the voltage dependence of Ca²⁺-permeable α-amino-3-hydroxy-5-methyl-4-

isoaxazolopropionic acid receptors (CP-AMPARs) is opposite to that of NMDARs (2, 3). Because CP-AMPARs are blocked by cytoplasmic polyamines upon depolarization (4, 5), maximal Ca²⁺ influx occurs when the membrane potential is relatively negative. LTP dependent on CP-AMPARs occurs in interneurons of the spinal cord and amygdala (6, 7), but its postsynaptic voltage dependence has not been explored. In hippocampal interneurons, CP-AMPARs have been implicated in long-term depression (8–10), and contribute to synaptic Ca²⁺ transients, especially in the stratum oriens/alevus (11). Many interneurons in the oriens/alevus also show NMDAR-independent LTP (12). We therefore looked for associative LTP in these cells, while recording with the gramicidin perforated patch technique to preserve intracellular polyamines (13).

Stimulation of pyramidal cell axon collaterals in the alveus evoked monosynaptic excitatory postsynaptic potentials (EPSPs) subthreshold for evoking action potentials. After recording a baseline, we paired high-frequency burst (HFB) stimulation (five pulses at 100 Hz, repeated 20 times) with stimulation of a second, suprathreshold, alveus pathway. “In-phase” associative pairing (phase difference Δφ = 0°) failed to elicit associative LTP in either pathway (n = 7; Fig. 1, A and B). In a further set of experiments, we alternately stimulated two weak pathways, and then delivered HFBs to both pathways antiphase (Δφ = 180°). This evoked a persistent increase in EPSP initial slope in one or both pathways in all cells (n = 7; Fig. 1, C and D). LTP was elicited even when HFB stimuli were delivered to only one weak pathway (n = 7; Fig. 1, E and F). Thus, LTP at excitatory synapses on interneurons in the oriens/alevus is prevented by associative pairing, in direct contrast to NMDAR-dependent LTP (1).

Can direct manipulation of the postsynaptic membrane potential similarly gate LTP induction? We delivered HFBs to one pathway
coinciding with the trough (somatic voltage: −90 mV) of an imposed 4-Hz sinusoidal somatic membrane potential oscillation. HFBs were then delivered to the other pathway coinciding with the depolarizing phase. In 8 out of 11 cells, pairing with hyperpolarization, but not with depolarization, resulted in LTP (Fig. 2, A and B). One cell showed the opposite behavior, and the other two showed no effect of either pairing (Fig. S1). Single alveus stimuli in phase with maximum hyperpolarization (100 times) also induced LTP (n = 10; Fig. 2C), but pairing with depolarization was ineffective (Fig. 2D). Thus, even low-frequency stimulation can trigger LTP if interneurons are hyperpolarized.

Because the induction requirements for LTP in most interneurons in the oriens/alveus are diametrically opposite to Hebb’s postulate (14, 15), we refer to it as “anti-Hebbian.” We tested the same LTP induction protocols in interneurons in the stratum radiatum. Hebbian LTP could be elicited in about half of these cells, many of which mediate feedforward inhibition (16), whereas pairing either HFB or low-frequency stimuli with hyperpolarization was uniformly unsuccessful (figs. S1 and S2). Anti-Hebbian LTP is thus characteristic of excitatory synapses made by local pyramidal cells on interneurons in the oriens/alveus but not of Schaffer collateral synapses on interneurons in the stratum radiatum.

Can differences in synaptic glutamate receptors explain whether Hebbian, anti-Hebbian, or no LTP is elicited? When interneurons in the oriens/alveus were recorded in whole-cell voltage clamp [with γ-amino butyric acid (GABA) receptors blocked, and with spermine included in the pipette solution], synaptic AMPARs activated by alveus stimulation were generally strongly rectifying (Fig. 2E), consistent with expression of CP-AMPARs (17). Furthermore, only small NMDAR-mediated synaptic currents were detected at a positive holding potential, consistent with low synaptic expression of the NR1 subunit (17).

We tested interneurons in the oriens/alveus, recorded in perforated patch mode, with a further anti-Hebbian protocol High-frequency stimulation of one alveus pathway (100 Hz, 100 pulses, delivered twice) paired with hyperpolarization, with NMDARs blocked, elicited LTP in 25 out of 31 cells (Figs. 3A and 4C). We repatched 11 of these cells in whole-cell voltage-clamp mode and found pronounced synaptic AMPAR rectification in every cell where anti-Hebbian LTP was evoked. The rectification index did not differ detectably between control and potentiated pathways (Fig. 3B), yielding no evidence for an LTP-related change in the permeability of synaptic AMPARs to Ca2+ (18).

In contrast, repatched interneurons in the stratum radiatum generally showed nonrectifying AMPARs and a large NMDAR-mediated component of Schaffer collateral-evoked synaptic currents (fig. S2) (9). The anti-Hebbian LTP induction protocol was successful in only 2 out of 20 cells in the stratum radiatum. AMPARs at Schaffer collateral synapses on 11 cells (none of which showed anti-Hebbian LTP) were nonrectifying (fig. S3).

Anti-Hebbian LTP thus typically occurs at synapses on interneurons in the oriens/alveus equipped with rectifying CP-AMPARs. Are these a uniform subgroup? Seven interneurons were regular-spiking oriens-lacunosum molecular (O-LM) cells (Fig. 3C, fig. S4), which mediate feedback inhibition of the apical dendrites of pyramidal neurons (19). Twelve other interneurons had horizontal dendrites and electrophysiological properties typical of O-LM cells, but axon visualization was incomplete (fig. S5). Anti-Hebbian LTP, however, also occurred in 17 out of 24 fast-spiking interneurons in the strata oriens or pyramidale, including one anatomically confirmed axo-axonic and two basket cells, which are innervated by CA1 pyramidal cells and target their perisomatic area. Seven other cells could not be classified.

**Fig. 1.** Associative pairing precludes LTP in interneurons in the stratum oriens/alveus. (A) Left: Schematic illustrating in-phase high-frequency burst (HFB) stimulation of weak and strong alveus pathways (filled and open symbols, respectively). Sample traces (1 to 5) show action potentials evoked by pairing in one cell. Right: Baseline-normalized EPSP initial slopes (mean ± SEM). (B) Top: Averaged EPSPs recorded before (blue) and after (red) pairing in one cell, showing the interval used to measure the initial slope. Bottom: Baseline-normalized EPSP initial slopes (25 min after pairing) in the two pathways, plotted against one another. (C) Antiphase pairing of two weak pathways induced LTP in seven out of seven cells. Sample traces (left) are from one cell. (D) EPSPs before and after pairing and summary of results, plotted as in (B). (E) Burst stimulation of one pathway also induced LTP. AMPA/kainate receptors were blocked at the end of the experiment (NBQX), to verify that EPSP initial slopes were not contaminated by monosynaptic inhibition. (F) Effect of HFB stimulation of one pathway (weak 1), plotted as for (B) and (D). Traces (top) also show the effect of NBQX. Data in (C) (right) and (E) (right) are shown as the mean ± SEM. Vm, membrane potential.
Anti-Hebbian LTP is, however, rare at Schaffer collateral synapses on interneurons in the stratum radiatum, which generally mediate feedforward inhibition and express nonrectifying receptors (Fig. 3, D and E). Synaptic responses evoked by stratum radiatum stimulation in fast-spiking interneurons in the stratum pyramidale, however, had strongly rectifying AMPARs and a small NMDA component, and the Hebbian LTP induction protocol was uniformly unsuccessful ($n = 4$; fig. S6).

Does rectification of CP-AMPARs fully explain the anti-Hebbian nature of LTP in interneurons in the oriens/alveus? We first verified that AMPA/kainate receptors are necessary for induction, by pairing HFS with postsynaptic hyperpolarization while AMPA/kainate receptors were blocked with 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline (NBQX, 5 μM): After washout of the antagonist, EPSPs in the tetanized and control pathways recovered to the same extent ($n = 7$; Fig. 3F). We then explored systematically the voltage dependence of LTP. In five cells in the oriens/alveus where anti-Hebbian LTP was evoked in one alveus pathway, subsequent pairing of the other pathway with depolarization only evoked short-lived post-tetanic potentiation (Fig. 4A). In six other cells, pairing the second pathway with hyperpolarization elicited robust LTP in all cases (Fig. 4, B and C). We then adapted this experimental design to explore the effect of manipulating the rectification properties of CP-AMPARs. Having demonstrated anti-Hebbian LTP in one pathway, we repatched the interneuron in whole-cell mode either with or without spermine in the pipette solution. Following a short baseline recording ($\leq 7$ min from patch rupture), we then paired HFS of the second pathway either with depolarization ($+20$ mV) or with hyperpolarization ($−90$ mV). When spermine was omitted, pairing with depolarization evoked LTP in five out of five cells (Fig. 4D), consistent with $\text{Ca}^{2+}$ influx via CP-AMPARs rendered non-rectifying by removal of polyamines (4). In contrast, HFS paired with depolarization failed to elicit LTP in five cells that were repatched with a spermine-containing pipette (Fig. 4E). In five other interneurons repatched with a spermine-containing solution, pairing HFS of the second pathway with hyperpolarization to $−90$ mV evoked LTP (Fig. 4F).

Polyamine-mediated rectification of AMPARs (and/or kainate receptors) thus explains the voltage dependence of LTP induction in these interneurons and reconciles our results with previous reports that a Hebbian protocol induces LTP in interneurons in the oriens/alveus when recorded with a polyamine-free whole-cell pipette solution (12). Also consistent with these reports, blockade of group I metabotropic glutamate receptors prevented LTP induction in interneurons with horizontal dendrites in the oriens/alveus (fig. S7). Finally, we looked for evidence that anti-Hebbian LTP is accompanied by an increase in glutamate-release probability (12), by applying extracellular polyamines, which also block CP-AMPARs in a use-dependent manner (20). After inducing anti-Hebbian LTP in one pathway, bath perfusion of $N$-(4-hydroxyphenylpropanoyl)-spermine (5 to 10 μM) caused a progressive decrease in EPSP initial slope, which was significantly faster in the paired than in the control pathway ($n = 7$; fig. S8). Given that anti-Hebbian LTP did not alter AMPAR rectification (Fig. 3B), this result is consistent with presynaptic expression.

Anti-Hebbian LTP may play distinct roles in neurons that show characteristic phase relationships in different network states (21, 22). During sharp-wave ripples, O-LM cells are typically silent, while many of their input pyramidal neu-

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**Fig. 2.** Postsynaptic membrane potential gates anti-Hebbian LTP induction. (A) LTP was evoked by pairing presynaptic stimulation with the hyperpolarizing but not the depolarizing phase of an imposed sinusoidal membrane potential oscillation. Left: Schematic and sample membrane potential traces during pairing in one cell (five sweeps superimposed for each pairing protocol). Right: Baseline-normalized EPSP initial slopes in eight cells showing LTP after anti-Hebbian pairing of HFS stimulation of one pathway with hyperpolarization. Subsequent Hebbian pairing of the other pathway with depolarization was ineffective. AMPA/kainate receptors were blocked at the end of the experiment (NBQX). Data are shown as the mean ± SEM. (B) Averaged EPSPs in one cell taken at the times indicated and after NBQX addition. Top: Anti-Hebbian pairing. Bottom: Hebbian pairing. (C) LTP was induced by pairing single stimuli at 5 Hz with hyperpolarization. Left: Sample traces during pairing. Right: Averages of all cells tested. Data are shown as the mean ± SEM. (D) Pairing with depolarization failed to induce LTP. Left: Sample traces during pairing. Right: Averages of all cells tested. Data are shown as the mean ± SEM. (E) Repatched interneurons recorded in whole-cell voltage-clamp mode show rectifying AMPARs and a negligible NMDAR-mediated component (GABA receptors blocked). Traces: Averaged EPSCs at $+60$ and $−60$ mV, showing the times at which the two components were measured. Bottom: current-voltage (I-V) relation of AMPAR-mediated EPSCs in six repatched interneurons (left). I-V relation for the NMDAR-mediated component, normalized by the AMPA EPSC at $−60$ mV (right).
rons fire at high frequency (21), possibly satisfying the induction conditions for anti-Hebbian LTP. Binding of pyramidal neurons to a spatial map may occur during periods of high-frequency firing (23), similar to sharp-wave ripples. In contrast, during theta activity, which is associated with exploratory behavior (24), O-LM cells fire in phase with pyramidal cells (21) and may contribute to this oscillation through phase-locked dendritic inhibition (25). Anti-Hebbian LTP induced during ripples may therefore result in a long-term alteration of pyramidal cell excitation of O-LM cells, which persists during theta activity, and may therefore contribute to spatial memory formation, the early stages of which have been shown to withstand NMDAR blockade (26).

References and notes
Intracellular polyamines determine the voltage dependence of anti-Hebbian LTP. (A) Postsynaptic depolarization prevents LTP induction. Data from cells recorded in perforated patch mode, showing LTP induced by pairing high-frequency stimulation (HFS) of one pathway with hyperpolarization (top, “anti-Hebbian”), and failure to induce LTP by pairing the other pathway with depolarization (bottom, “Hebbian”). NMDARs were blocked throughout. Data are the mean ± SEM. (B) In six other cells, the second pathway was subsequently paired with hyperpolarization, yielding anti-Hebbian LTP in all cases. Data are the mean ± SEM. (C) Baseline-normalized EPSP slopes plotted against one another 20 min after anti-Hebbian (left) and Hebbian (right) pairing. Insets: Sample membrane potential traces during pairing. (D) Anti-Hebbian LTP was first induced in one pathway (left, filled symbols). The interneuron was then repatched in whole-cell mode with a polyamine-free pipette solution. HFS delivered to the second pathway (right, open symbols) paired with postsynaptic depolarization (+20 mV) induced LTP. Top: Voltage (left) and current (middle, with seal resistance test artefacts) traces during pairing, and one O-LM cell identified among five interneurons in the sample (right; scale bar: 200 μm). (E) Intracellular spermine blocked LTP induction in the second pathway when paired with depolarization. Top: As in (D). (F) LTP was induced with intracellular spermine when paired with hyperpolarization (−90 mV). Top: As in (D) and (E). Data in (D), (E), and (F) (bottom panels) are shown as the mean ± SEM.

13. Materials and methods are available as supporting material on Science Online.
27. Animal procedures followed the Animals (Scientific Procedures) Act 1986. Supported by the Wellcome Trust, the Academy of Finland, and the Medical Research Council. We are grateful to M. C. Walker and K. Volynski for comments, and to J. D. B. Roberts for help with histological processing.

Supporting Online Material
www.sciencemag.org/cgi/content/full/315/5816/1262/DC1
Materials and Methods
Figs. S1 to S8
References
13 November 2006; accepted 1 February 2007
10.1126/science.1137450
Supporting Online Material

Materials and Methods

Three to four week old male Sprague-Dawley rats were sacrificed according to the Animals (Scientific Procedures) Act 1986. Hippocampal slices (300 – 350 µm thick) were visualized with infra-red differential interference contrast and epifluorescence imaging. A cut was made between CA1 and CA3 to prevent propagation of recurrent excitation in Schaffer collaterals. The perfusion medium contained (in mM): NaCl (119), KCl (2.5), MgCl\(_2\) (1.3), CaCl\(_2\) (2.5), NaHCO\(_3\) (25), NaH\(_2\)PO\(_4\) (1), glucose (11), equilibrated with 95% O\(_2\): 5% CO\(_2\) (pH 7.4, 31 – 32\(^\circ\) C). The GABA receptor blockers picrotoxin (100 µM) and CGP52432 (5 µM) were routinely added to the solution (except in Figs. 1 and 2 and figs. S1 and S6).

Electrical stimuli (50 µs, 50 – 500 µA) were delivered via bipolar stainless steel electrodes in the alveus, with a 15 – 20 s inter-trial interval during baseline and after LTP induction. Cells were recorded with a Multiclamp 700 amplifier (Molecular Devices).

Perforated patch recordings were made with 10 – 15 MΩ pipettes containing gramicidin (100 µg/ml) in a solution containing (in mM): K-gluconate (145), NaCl (8), KOH-HEPES (20 – 25), EGTA (0.2), and QX-314 Br (5) (pH 7.2, 295 mOsm). The electrode tip was filled with gramicidin-free solution. Series resistance was continuously monitored throughout the experiment, and recordings in bridge balance mode were started when it was <100 MΩ. Depolarizing currents were intermittently injected to evoke action potentials to verify patch integrity. In some experiments QX-314 was omitted and 20 µM Alexa Fluor 488 was included, and the neuron was imaged with epifluorescence to monitor dye penetration. If the patch ruptured spontaneously the experiment was discontinued.

Evoked EPSPs were recorded from the resting membrane potential (liquid junction potential corrected), low-pass filtered (5 Hz) and acquired at 20 kHz on PC for off-line analysis (LabView, National Instruments). In some experiments EPSPs were recorded during a brief (500 ms) hyperpolarizing step (5 – 10 mV) to avoid action potential generation. The initial slope (range 3 – 7 ms from onset) of the EPSPs was measured, and in the LTP experiments all EPSPs were visually
verified to restrict attention to monosynaptic excitatory inputs. (Analyzing the peak slope during the same interval gave similar results.)

In the experiments illustrated in Fig. 1 the baseline EPSP amplitude of the ‘weak’ pathways was 3.1 ± 1.7 mV (Fig. 1A), 2.8 ± 0.2 mV (Fig. 1C), and 3.4. ± 0.7 mV (Fig. 1E). High-frequency burst (HFB) stimulation consisted of brief 5-pulse trains at 100 Hz. Five HFBs were delivered at 4 – 5 Hz, and this was repeated 4 times, with 10 s pauses between groups of HFBs (100 pulses in total). During anti-phase pairing (Fig. 1C) the somatic membrane potential was -58.6 ± 3.0 mV, compared to a resting value of -65.2 ± 1.6 mV. For low-frequency stimulation the same total number of stimuli (100) was given at 1 or 5 Hz, as indicated. High-frequency tetanic stimulation (HFS) consisted of 100 pulses at 100 Hz, delivered twice with a 10 s pause.

For the experiments illustrated in Fig. 2 the imposed somatic voltage oscillation was designed to give a trough membrane potential of -90 mV, which is likely to overestimate the dendritic hyperpolarization because of electrotonic attenuation (1).

Whole cell voltage-clamp recordings were made with a solution containing (in mM): Cs-gluconate (117.5), CsCl (17.5), KOH-HEPES (10), BAPTA (10), NaCl (8), Mg-ATP (2), GTP (0.3), spermine tetrahydrochloride (0.1) and QX-314 Br (5) (pH 7.2, 290 mOsm). Biocytin or neurobiotin (0.4%) was included in most experiments. Cells showing unstable series resistance or holding current were rejected. Because NMDAR-mediated signaling rapidly washes out when interneurons in stratum radiatum are recorded in whole cell mode (2) we measured the I-V relationship for NMDAR-mediated EPSPs within 5 minutes of breaking in. The AMPAR-mediated EPSC rectification index was obtained by dividing the amplitude of the EPSC recorded at +60 mV by that predicted from linear extrapolation of the current-voltage (I-V) relationship measured at negative potentials. For this extrapolation, the regression line was taken from EPSCs (mean of ≥ 4 at each point) at -90mV, -60 mV, and -30 mV. The NMDA/AMPA ratio was calculated by dividing the amplitude of the late outward NMDAR-mediated EPSC at +60 mV by the amplitude of the early inward AMPAR-mediated EPSC at -60 mV. The early and late time points were ≤ 5ms and 50 ms from EPSC onset, respectively. (We have not discriminated between AMPA and kainate receptors with selective blockers in this study. Therefore we cannot exclude a contribution of Ca²⁺-permeable kainate receptors, although their slower kinetics (3) make it unlikely that they contribute substantially to the early EPSC used to measure rectification.) For the whole-cell current-clamp experiments illustrated
in Fig. 4, K-gluconate was used instead of Cs-gluconate, and the spermine concentration, where added, was 1 mM. Series resistance (<20 MΩ) was monitored throughout the experiment using a -5 or -10 mV step command. In Fig. 4, seal test steps (-10 mV) were run also during the pairing protocol (see insets in Fig. 4D - F).

Although repetitive synaptic activity attenuates polyamine blockade of CP-AMPARs (4), HFB stimulation did not prevent rectification of alveus-evoked EPSCs in 5 interneurons in stratum oriens when recorded with a whole-cell pipette solution containing as little as 100 µM spermine (data not shown). Endogenous polyamines have been estimated to be equivalent to this concentration or higher (5, 6).

For cell identification, a whole-cell recording was obtained from the same cell with a pipette containing 0.4% neurobiotin, biocytin, or biocytin conjugated to the fluorescent dye Alexa Fluor 488. Slices were fixed overnight in 4% paraformaldehyde at 4 C. After permeabilization in 0.1% TritonX-100, slices were incubated in 0.1% streptavidin-Cy3 conjugate, mounted in DABCO anti-fading medium. Cells were first imaged with a confocal microscope to record the dendritic and axonal patterns, whereupon the slices were incubated in a solution of avidin-biotinylated horseradish peroxidase (HRP) complex in Tris-buffered (TB) saline, containing 0.3% Triton. Peroxidase activity was visualized by incubation for 5 min in 0.05% 3,3′-diaminobenzidine tetrahydrochloride (DAB) in TB (pH 7.6) followed by another 10 min in DAB containing 0.01% H₂O₂. After more extensive washing, first in TB, then in 0.1 M phosphate buffer (PB), slices were treated with 1% OsO₄ in 0.1 M PB for 1 hour before they were washed in PB, dehydrated, and finally permanently mounted in Durcupan resin on glass slides under a coverslip.

Data are shown as mean ± s.e.m., normalized by baseline values. Significance was analyzed with Student’s paired t-test. In order to estimate the magnitude of LTP the change in EPSP initial slope for the paired pathway was corrected for minor drift in the unpaired control pathway, according to the formula LTP = (paired2/paired1) ÷ (control2/control1), where the subscripts 1 and 2 refer to EPSP slopes measured before and after pairing, respectively. This gave the following values. Fig. 2A: 79 ± 15 % increase in EPSP initial slope when paired with hyperpolarization (n = 8), and 3 ± 10 % when paired with depolarization. Fig. 2C (all 10 cells paired with hyperpolarization): 36 ± 12 %. Fig. 2D (all 10 cells paired with depolarization): -6 ± 5 %. Fig. 3A (25 cells exhibiting LTP, measured 20-25 min after pairing): 64 ± 6 % (the other 6 cells showed no change). Fig. 4A: 87 ± 11
% (20 minutes after pairing) when paired with hyperpolarisation, and 13 ± 6 % when paired with
depolarisation. Fig. 4B: 57 ± 7 % (1st pathway paired with hyperpolarisation), 12 ± 6 % (2nd
pathway paired with depolarization), and 52 ± 6 % (paired with hyperpolarisation). Fig 4D: 42 ± 3
% (first pathway paired in perforated patch mode), 55 ± 5 % (second pathway paired in whole cell
mode). Fig. 4E: 67 ± 10 % (1st pathway paired in perforated patch mode). Fig. 4F: 83 ± 7 % (1st
pathway in perforated patch mode), 81 ± 5 % (second pathway in whole cell mode).
Lamsa et al. Fig. S1

**A1**

![Diagram A1](image)

**A2**

![Diagram A2](image)

**A3**

![Diagram A3](image)

**A4**

![Diagram A4](image)

**B**

Anti-Hebbian and Hebbian LTP tested

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**C1**

![Diagram C1](image)

**C2**

![Diagram C2](image)

**C3**

![Diagram C3](image)

**D**

Anti-Hebbian and Hebbian LTP tested

![Diagram D](image)

**E1**

![Diagram E1](image)

**E2**

![Diagram E2](image)

**E3**

![Diagram E3](image)

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5 Hz • "anti-Hebbian"

n=10 cells

P < 0.01

"Hebbian"

"anti-Hebbian"

"Hebbian"

5 Hz • "anti-Hebbian"

n=10 cells
Fig. S1. Anti-Hebbian LTP occurs at synapses made by pyramidal cell axons on interneurons in oriens-alveus but is rare at synapses made by Schaffer collaterals on interneurons in stratum radiatum.

(A) High-frequency (100 Hz) burst (HFB) stimulation of the alveus, paired with the trough of an imposed postsynaptic sinusoidal membrane potential oscillation (“anti-Hebbian pairing”), induced pathway-specific LTP in 8 out of 11 interneurons in stratum (s.) oriens-alveus. A1 Schematic showing arrangement of recording (perforated patch) and stimulating electrodes. A2 Time-course of EPSP initial slopes in both pathways (mean ± s.e.m., n = 8, replotted from Fig. 2). LTP was not seen when stimulation of the other alveus pathway was paired with postsynaptic depolarization. A3 Two interneurons did not exhibit any potentiation following either pairing protocol. A4 One interneuron exhibited LTP following Hebbian but not anti-Hebbian pairing. (B) Pie chart showing the proportion of interneurons in each class. (C) Anti-Hebbian HFB did not elicit LTP at Schaffer collateral synapses onto interneurons in stratum radiatum. C1 Schematic illustrating experimental design. Instead, 6/11 interneurons exhibited LTP following Hebbian pairing (C2). C3 The other five interneurons did not exhibit LTP with either protocol. (D) Summary pie chart. (E) Results of pairing single stimuli at 5 Hz with the trough (anti-Hebbian) or peak (Hebbian) of the somatic membrane potential oscillation in interneurons in s. radiatum. E1 Schematic showing experimental design. E2 Pairing with hyperpolarization (anti-Hebbian) failed to induce LTP (all 10 cells tested included in plot). E3 Pairing with depolarization (Hebbian) induced LTP (all cells included).

Although the term ‘anti-Hebbian’ has previously been used to describe long-term depression triggered by Hebbian pairing, we have used the term ‘anti-Hebbian LTP’ here to denote an increase in synaptic strength triggered by presynaptic activity and blocked by postsynaptic depolarization. It is induced by brief trains of presynaptic action potentials when the postsynaptic membrane potential is at rest, or by single action potentials when the membrane is hyperpolarized. We are aware of only one example of LTP with an anti-Hebbian induction rule in cerebellar nuclei (7), although this depends on NMDARs and also requires a rebound depolarization following the pairing. In contrast, anti-Hebbian LTP reported here depends on rectifying CP-AMPARs.
Lamsa et al. Fig. S2

A1. Perf. patch

A2. 1 Hz ■ "Hebbian"  

A3. 1 Hz ■ "Hebbian"  

B1. NMDA/AMPA ratio unpaired pathway

B2. NMDA/AMPA ratio paired pathway

B3. NMDA/AMPA ratio unpaired pathway

C1. Perf. patch

C2. 1 Hz ■ "Hebbian"  
P<0.01  

C3. 1 Hz ■ "Hebbian"  

D1. SC1  

D2. SC2  

D3. AMPA  

D4. NMDA

D5. NMDA/AMPA ratio unpaired pathway

K90952

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Fig. S2. Hebbian LTP occurs in interneurons with non-rectifying AMPARs and large NMDAR-mediated EPSCs.

(A) Pairing 1 Hz stimulation with depolarizing voltage commands failed to evoke LTP in all 12 tested interneurons in *s. oriens/alveus*. (LTP was elicited in one displaced pyramidal cell identified following re-patching in whole cell mode and *post hoc* visualization, which has been excluded.) A1 Schematic showing experimental design, and sample trace obtained in one cell during pairing (*I_m*), showing a train of escape currents. A2 Mean EPSP initial slope for 12 cells tested (pairing at time indicated by arrow). A3 Baseline-normalised EPSP initial slopes in paired and control pathways plotted against one another. Insets: averaged EPSPs in both pathways, recorded at times indicated in one cell. (B) Re-patched interneurons mainly showed highly rectifying synaptic AMPARs and a small NMDAR-mediated component. B1 Rectification indices for paired and unpaired pathways in 6 re-patched interneurons. B2 NMDA/AMPA ratio in 5 cells. (The NMDAR-mediated component could not be reliably measured in one further cell.) Sample traces: EPSCs measured at +60 mV and -60 mV, respectively (scale-bars 50 pA / 50 ms), in both pathways. B3 The re-patched interneurons were visualized *post hoc*. Three were identified as O-LM cells, one of which is shown with axon in blue and somatodendritic structures in red (Scale: 200 µm). Three further cells had horizontal dendrites but axons were not visualized. (C) Hebbian LTP was induced in 17 out of 37 interneurons in *stratum radiatum*. C1 Schematic showing experimental design and sample traces during pairing in one cell. C2 Top: Mean EPSP initial slope for 17 cells showing LTP of the paired pathway (filled symbols). Bottom: 20 cells not showing LTP. C3 EPSP slopes plotted as in A3. Gray symbols: cells showing pathway-specific LTP. Inset; averaged EPSPs before (-10 min) and after (20 min) LTP induction in one cell. (D) Re-patched interneurons in *stratum radiatum* exhibiting Hebbian LTP had non-rectifying AMPARs and large NMDAR-mediated EPSCs. D1 AMPAR-mediated EPSC *I-V* relationship obtained from 9 re-patched cells expressing Hebbian LTP. D2 EPSCs in the potentiated and un-paired pathways show similar rectification profiles. Gray symbols: cells exhibiting Hebbian LTP. D3 *I-V* relationship of the NMDAR-mediated component in cells showing Hebbian LTP. D4 NMDA/AMPA ratio. The symbols for the cells showing LTP fall above the line of identity, consistent with a relatively selective increase in the AMPA component. Inset; averaged EPSCs at -60 and +60 mV. Scale-bars 50 pA / 50 ms. D5 Soma and dendrites of an interneuron showing Hebbian LTP (scale: 200 µm). The axon was not recovered and further identification was therefore not possible. GABA receptors were blocked in all experiments.
Lamsa et al. Fig. S3

A

Perf. patch

SC1

s.rad

SC2

HFS • “Hebbian”

APV (100 µM)

EPSP slope (norm.)

n=20 cells

Time (min)

B

HFS • “anti-Hebbian”

APV (100 µM)

EPSP slope (norm.)

n=20 cells

Time (min)

C

EPSP (untetanized pathway)

EPSP (tetanized pathway)

n=40

D

SC1

s.rad

SC2

whole cell

EPSC rectification index

n=11 cells

20 pA

50 ms
Fig. S3. NMDAR-independent LTP is rare at Schaffer collateral synapses on interneurons in *s. radiatum*.

(A) Top: Schematic showing experimental design. SC1, SC2: Schaffer collateral pathways. Bottom: Pairing high-frequency afferent stimulation (100 Hz 1s, twice) with postsynaptic depolarization in the presence of the NMDAR antagonist APV (100 µM) failed to induce pathway-specific potentiation lasting >10 min in any of 20 cells studied (filled symbols). Open symbols indicate the unpaired control pathway. Right: averaged EPSPs in one cell in both pathways before and after pairing. (B) Results of pairing high-frequency stimulation with postsynaptic hyperpolarization in 20 cells. Right: traces from one cell. (C) Baseline-normalised EPSP slopes in the tetanized and control pathways plotted against one another 15 min following the pairing. NMDAR-independent LTP was elicited in only two out of 40 attempts (gray symbols). The 40 pairings were performed in 17 interneurons in *stratum radiatum* where two pathways were tested, and 6 cells where only one pathway was tested, with either depolarization or hyperpolarization. (D) Re-patching 11 interneurons, none of which exhibited LTP, revealed weakly or non-rectifying AMPAR-mediated EPSCs. The histogram shows the distribution of the EPSC rectification indices in all 22 pathways. Inset: averaged EPSCs from one re-patched interneuron, recorded at -60mV and +60 mV. The two interneurons that showed anti-Hebbian LTP were not successfully re-patched. However, rectifying AMPAR-mediated EPSCs were recorded in 4 fast-spiking interneurons in *stratum pyramidale* when stimulating in *stratum radiatum* (see fig. S6). GABA receptors were blocked in all experiments.
Fast spiking (FS) interneuron

Rebounding Regularly-Spiking Nonpyramidal (R-RSNP) neuron

Non-Rebounding Regularly-Spiking Nonpyramidal (NR-RSNP) neuron

Rapidly Adapting Regularly-Spiking Nonpyramidal (RA-RSNP) neuron

Lamsa et al. Fig. S4A-D
Delayed-Spiking (DS) interneuron

Burst-Spiking Nonpyramidal (BSNP) neuron

Irregular-Spiking (IS) interneuron

Lamsa et al. Fig. S4E-G
Interneurons were divided into 7 categories according to their passive and firing properties: regular-spiking non-pyramidal cells (RSNP), classified further as rebounding (R-), non-rebounding (NR-), and rapidly adapting (RA-) RSNPs; burst-spiking nonpyramidal cells (BSNP); fast-spiking interneurons (FS); irregularly-spiking interneurons (IS); delayed spiking interneurons (DS). (A) FS interneurons. A1 FS interneurons were either non-rebounding (left) or rebounding (right). Rebounding interneurons generated action potentials (arrow) on release from hyperpolarizing current injection (to -90 mV). Interneurons in this class were recorded in stratum oriens and s. pyramidale. A2 These cells had a high maximal spiking frequency and small reduction in the spiking frequency when comparing initial (0-100 ms) and later (400-500 ms) periods. (B) R-RSNP cells. B1 Representative R-RSNPs in stratum oriens (left) and stratum radiatum (right). Rebounding action potentials indicated by arrow. B2 RSNPs showed spike frequency adaptation during maximal spiking. (C) NR-RSNP cells. C1 NR-RSNPs in stratum oriens (left) and stratum radiatum (right). C2 as above. (D) RA-RSNP cells. D1 RA-RSNPs in stratum oriens and stratum radiatum. These cells showed initially regular spiking behaviour, which stopped after a few hundred milliseconds during maximal spiking. D2 as above. (E) BSNP cells. E1 Representative BSNP-type interneurons recorded in both strata. These cells characteristically generated high-frequency bursts of action potentials (≥3) on release from hyperpolarizing current injection. E2 as above. (F) IS cells. F1 In IS interneurons spiking was irregular and intermittent even during their maximal spiking frequency. F2 as above. (G) DS cells. G1 DS interneuron recorded in stratum radiatum. G2 as above.
Lamsa et al. Fig. S5

Max. firing

Firing adaptation

Delay to spike

AHP

Rin

Em

Tau

Vsag

O-LM
Horizontal
Fast spiking
Other

0 100 200 300 (Hz)

0 20 40 60 80 (%)

0 40 80 120 160 (ms)

0 5 10 15 20 25 (mV)

0 200 400 600 800 (MΩ)

-70 -60 -50 -40 (mV)

0 10 20 30 40 50 (ms)

0 5 10 15 20 25 30 (mV)
Fig. S5. Electrophysiological properties of interneurons showing anti-Hebbian LTP.

Box plots comparing active and passive membrane properties of re-patched interneurons and all fast-spiking cells that showed anti-Hebbian LTP (electrophysiological properties recorded in perforated patch). Cells were categorized as: O-LM (anatomically identified O-LM cells, n = 7); horizontal (cells with horizontal dendrites similar to those of the identified O-LM cells, but with insufficient axon visualization, n = 12); fast spiking (fast-spiking interneurons, which characteristically showed little spike frequency adaptation, n = 17); other (anatomically unidentified, firing pattern not fast-spiking, n = 7). The data suggest that anti-Hebbian LTP is not restricted to O-LM and fast-spiking interneurons contributing to the feedback inhibitory circuitry.

Maximal firing frequency was taken from the first 100 ms during maximal action potential firing generated by a >500 ms depolarizing step. Firing adaptation (%) was obtained by comparing the initial 0-100 ms and subsequent interval 400-500 ms following injection of a current giving a maximal firing frequency. The delay to spike represents an average latency to the first spike for three or more depolarizing steps slightly above the firing threshold. Afterhyperpolarization (AHP) amplitude was measured from the first action potential initiated during the delay-to spike test. Input resistance (Rin) was measured using a -100 pA (1 s) current pulse. Em, resting membrane potential. Membrane time constant (Tau) was determined from the negative slope of a -10 mV step at resting membrane potential. V-sag was measured from a hyperpolarizing step to -90 mV.
Lamsa et al. Fig. S6

A1: IN perf. patch
PC whole cell
s.pyr

A2: IN
PC
IN perf. patch

A3: 60 mV
10 pA
5 ms

IN whole cell

B1: s.rad
s.pyr
alveus

B2: SC-EPSP (paired Hebbian)
alveus-EPSP (unpaired)

C1: IN whole cell
s.rad
s.pyr
alveus

C2: SC-EPSC
alveus-EPSC

500 ms
220 Hz
230 Hz

10 mV
-10 mV
-60 mV
+60 mV
220 Hz
230 Hz

25 pA
50 ms

220 Hz
230 Hz

220 Hz
230 Hz

220 Hz
230 Hz

220 Hz
230 Hz
Fig. S6. Evidence for target-cell dependent properties of synapses made by Schaffer collaterals on inhibitory interneurons.

Excitatory currents evoked by stimulating in stratum radiatum (to activate Schaffer collaterals) and in the alveus (to stimulate CA1 pyramidal cell collaterals) were compared in fast-spiking interneurons in stratum pyramidale. These cells contribute to both feed-forward and feedback inhibition (9). (A) Fast-spiking GABAergic interneurons (recorded in perforated patch mode in stratum pyramidale) were identified by demonstrating that a presynaptic action potential evoked an inhibitory postsynaptic current in a local pyramidal neuron. A₁ Schematic showing experimental design. A₂ Simultaneously recorded presynaptic voltage and postsynaptic current traces in one interneuron (IN) – pyramidal cell (PC) pair. A₃ The presynaptic cells showed a fast-spiking pattern in response to depolarizing current injection with very little firing adaptation. (B) Monosynaptic EPSPs were then elicited in the interneuron by stimulation in the alveus and in the stratum radiatum (B₁ schematics) to activate Schaffer collaterals and CA1 pyramidal cell axon collaterals, respectively. We then paired stratum radiatum stimulation with depolarization of the interneuron (100 Hz 1s, delivered twice). This failed to elicit LTP in any of four cells. B₂ Baseline-normalized EPSP initial slopes (25 min following pairing) in the two pathways, plotted against one another. (C) The interneurons were re-patched with whole cell (C₁). C₂ In contrast to Schaffer collateral-synapses onto interneurons in stratum radiatum, synaptic AMPARs in these cells showed strong rectification.
LY367385 (100 µM) + MPEP (10 µM)

EPSP slope (norm.)

Time (min)

HFS "anti-Hebbian"

LY367385 (100 µM) + MPEP (10 µM)

n=3 cells

P<0.05

100 Hz "anti-Hebbian"
Fig. S7. NMDAR-independent LTP in interneurons in *oriens/alveus* is sensitive to group I metabotropic glutamate receptor (mGluR) blockade.

Top: schematic showing arrangement of stimulation and recording electrodes. (Experiments were carried out in interneurons in *oriens/alveus* with horizontal dendrites.) NMDA and GABA receptors were blocked throughout. Summary plot: EPSP initial slope showing LTP induced by pairing tetanic stimulation of one pathway with hyperpolarization (filled symbols, top). The group I mGluR antagonists LY367385 (100 µM) and 2-Methyl-6-(phenylethynyl)-pyridine (MPEP, 10 µM) were subsequently washed in and the second pathway (open symbols, bottom) was paired as above. This failed to evoke any potentiation.

Although this result confirms that group I mGluRs are necessary for LTP induction in interneurons in *oriens/alveus* (10, 11), the effects of manipulating the postsynaptic voltage and polyamines show that they are not sufficient. We cannot exclude the possibility that the threshold for induction of LTP is modulated by the degree of activation of mGluRs: high frequency presynaptic stimulation, which may have preferentially activated perisynaptic group I mGluRs, was required to induce LTP when the postsynaptic membrane potential was near its resting value (Fig. 1C,D), but low frequency stimulation was sufficient when interneurons were hyperpolarized (Fig. 2C). Whether they play the same role in different types of interneurons exhibiting anti-Hebbian LTP, which show distinct patterns of immunoreactivity for group I mGluRs (12), remains to be determined.
Fig. S8

A. Perf. patch

B. HFS ■ “anti-Hebbian”

C. NHPP-SP (5-10 µM)

D. 1 min
10 min
20 min

EPSP slope (norm.)

EPSP slope (%)

Time (min)

Time (min)
Fig. S8. Anti-Hebbian LTP was accompanied by an increased sensitivity to extracellular polyamines.

(A) LTP was evoked in 7 cells recorded in perforated patch mode by pairing tetanic stimulation of one pathway (filled symbols) with hyperpolarization to -90 mV. (B) Summary plot showing pathway-specific LTP. (C) NHPP-spermine (5 – 10 µM) was subsequently added to the perfusion solution. This caused a gradual decrease in EPSP slope, which was more rapid in the LTP pathway than in the control pathway. EPSP initial slopes are shown normalized to the value immediately prior to application of NHPP-spermine. (D) EPSPs in potentiated and control pathways, showing a significantly greater attenuation of the potentiated EPSP. EPSPs are shown normalized by the peak amplitude prior to adding NHPP-spermine. Scale-bar: 50 ms. NMDA and GABA receptors were blocked in all experiments.

These results, taken together with evidence that LTP in interneurons in stratum oriens is associated with decreases in failure rate and short-term facilitation (10), imply a presynaptic expression mechanism. Using a similar experimental design we looked for evidence that CB1 receptors are involved, because these have been implicated in retrograde signaling at other synapses in the hippocampus (13). The CB1 receptor blocker AM-251 (20 µM) however failed to prevent anti-Hebbian LTP induction (143 ± 37 % increase in EPSP slope, n = 4, data not shown).
Supplementary references