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Spatial exploration induces a persistent reversal of long-term potentiation in rat hippocampus

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Experience-dependent long-lasting increases in excitatory synaptic transmission in the hippocampus are believed to underlie certain types of memory[1–3]. Whereas stimulation of hippocampal pathways in freely moving rats can readily elicit a long-term potentiation (LTP) of transmission that may last for weeks, previous studies have failed to detect persistent increases in synaptic efficacy after hippocampus-mediated learning[4–6]. As changes in synaptic efficacy are contingent on the history of plasticity at the synapses[7], we have examined the effect of experience-dependent hippocampal activation on transmission after the induction of LTP. We show that exploration of a new, non-stressful environment rapidly induces a complete and persistent reversal of the expression of high-frequency stimulation-induced early-phase LTP in the CA1 area of the hippocampus, without affecting baseline transmission in a control pathway. LTP expression is not affected by exploration of familiar environments. We found that spatial exploration affected LTP within a defined time window because neither the induction of LTP nor the maintenance of long-established LTP was blocked. The discovery of a novelty-induced reversal of LTP expression provides strong evidence that extensive long-lasting decreases in synaptic efficacy may act in tandem with enhancements at selected synapses to allow the detection and storage of new information by the hippocampus.

To study the effects of processing new information on the persistence of LTP in the hippocampus, we chose a task that is known to involve activation of this brain region, exploration of a new environment[8,9]. Familiar and novel environments consisted of two boxes that were clearly distinguishable on the basis of lighting (familiar, bright versus novel, dim; see Methods). We chose to use the darker box as the novel environment because of the well known preference of rats for dimly lit areas, thereby increasing the likelihood of exploratory behaviour and minimizing the likelihood of aversive reactions (such as neophobic behavioural freezing) in the new environment. Behavioural (reduced exploration; see Methods) and electrophysiological (reduced hippocampal activation; see below) evidence that this type of exploration was accompanied by the acquisition of information about the new environment was found when the animals were reintroduced to the box on the following days.

Experiments were carried out on freely behaving animals that had been habituated over a period of 2 weeks to the recording procedure and the familiar box. Once baseline synaptic transmission, as measured by the amplitude of the field excitatory postsynaptic potential (EPSP), was found to be stable over a period of at least 3 days, high-frequency conditioning stimulation was applied to the test pathway in order to induce LTP. The conditioning stimulation used in these studies (10 trains of 20 pulses at 200 Hz) was sufficient to elicit a relatively large potentiation of synaptic responses that remained constant over the subsequent 4-h recording period if the animals were kept in the familiar box (see Fig. 1a legend for

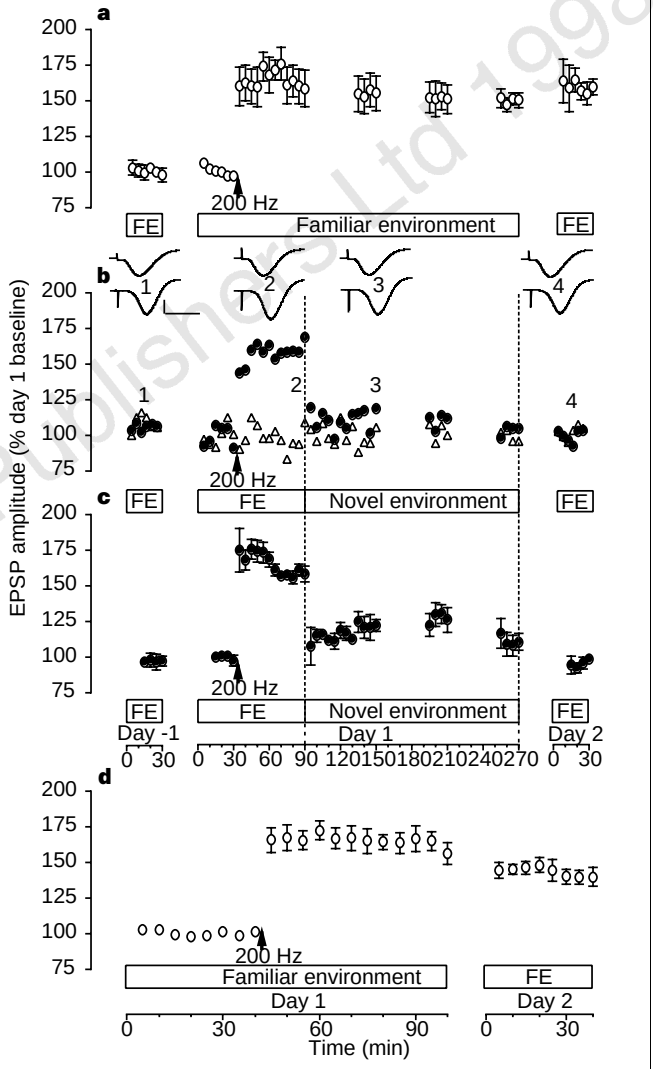

Figure 1 Exploration of a novel environment rapidly reverses LTP. **a**, High-frequency (200 Hz, arrow) stimulation induced stable LTP when induced and recorded in a familiar environment (FE). The amplitude of the field excitatory postsynaptic potential (EPSP) was significantly increased to 158.3 ± 12.9, 155.4 ± 12, 151.1 ± 9.9 and 159.7 ± 5.8% of baseline at 1, 2, 4 and 24 h after the conditioning stimulation (values are 5-min averages ± s.e.m., $P < 0.01$, $n = 9$). **b**, **c**, LTP was rapidly reversed when the animal was placed in a novel environment 1 h after the application of the high-frequency stimulation. Although the EPSP amplitude was increased at 1 h (159.8 ± 5.4), on introduction to the new environment (NE) synaptic responses returned towards baseline values, reaching 123.8 ± 4.2% at 2 h and 111.9 ± 5.9% at 4 h ($P > 0.05$ compared to baseline and $P < 0.01$ compared to potentiated level at 1 h or the level of LTP in controls; $n = 5$). LTP was still absent 24 h later when recorded in the familiar environment (98.5 ± 1.7%). **b**, Example of a two-pathway experiment. Test (black circles and lower traces) versus ipsilateral non-tetanized control pathway (white triangles and upper traces). Horizontal bar, 10 ms; vertical bar, 2 mV. **d**, Handling the animals by removing them from the familiar box to their home cage 1 h after inducing LTP had no significant effect on the magnitude of LTP when measured 24 h later in the familiar box (156 ± 7.6 and 139.8 ± 6.3% of baseline at 1 and 24 h after the conditioning stimulation; $P > 0.05$ compared to controls; $n = 5$).

statistics). The LTP was still present on the following day (Fig. 1a) and in some animals persisted for at least a week (data not shown). In marked contrast, when the animals were gently removed from their familiar recording box and placed in the novel box 1 h after the induction of LTP, the synaptic responses rapidly decreased to a level not significantly different from baseline levels (Fig. 1b, c). The reduction in the potentiated responses persisted for the subsequent 3-h recording period in the novel environment, far outlasting the initial increase in general locomotor activity that occurred as the animal explored the new box (<10 min). On the following day, the synaptic responses remained at baseline levels when the recordings were taken 20–40 min after the animals were placed in the familiar box while they were predominantly in a still, alert state (Fig. 1b, c). This confirms that a true reversal of LTP had occurred, rather than a transient state-dependent reduction. There was no change in the synaptic responses in a control pathway in animals that had a second stimulating electrode implanted ipsilaterally (Figs 1b, 2a). Furthermore, in separate control (non-tetanized) pathway experiments, there was no change in basal transmission after rats were moved from the familiar to the novel box (98.7 ± 3.9% at 40 min, $P > 0.05$ compared to baseline, 99.4 ± 3%, $n = 5$). Thus, the novel-environment-induced long-lasting reduction in synaptic transmission was restricted to the recently potentiated pathway. The possibility that the mild handling required to move the rats from one box to the other might be responsible for the reversal of LTP was discounted by lifting the animals from the familiar environment and placing them back in their home cage 1 h after the induction of LTP. Even though there was a transient increase in general motor activity on returning to their home cage, similar to that observed in the novel box, there was no significant change in the magnitude of LTP compared to the control animals (Fig. 1d).

An opaque perspex barrier was used to separate the unfamiliar box from the familiar box in subsequent experiments in order to avoid handling the animals at the time of introducing them to the novel environment. When this barrier was removed, the rats freely entered, explored and remained in the novel area. In animals that entered the novel box for the first time 1 h after LTP induction, a rapid reduction of synaptic transmission to baseline levels was observed which persisted while they remained in the novel environment for the next 3 h. This reduction was still present 24 h later

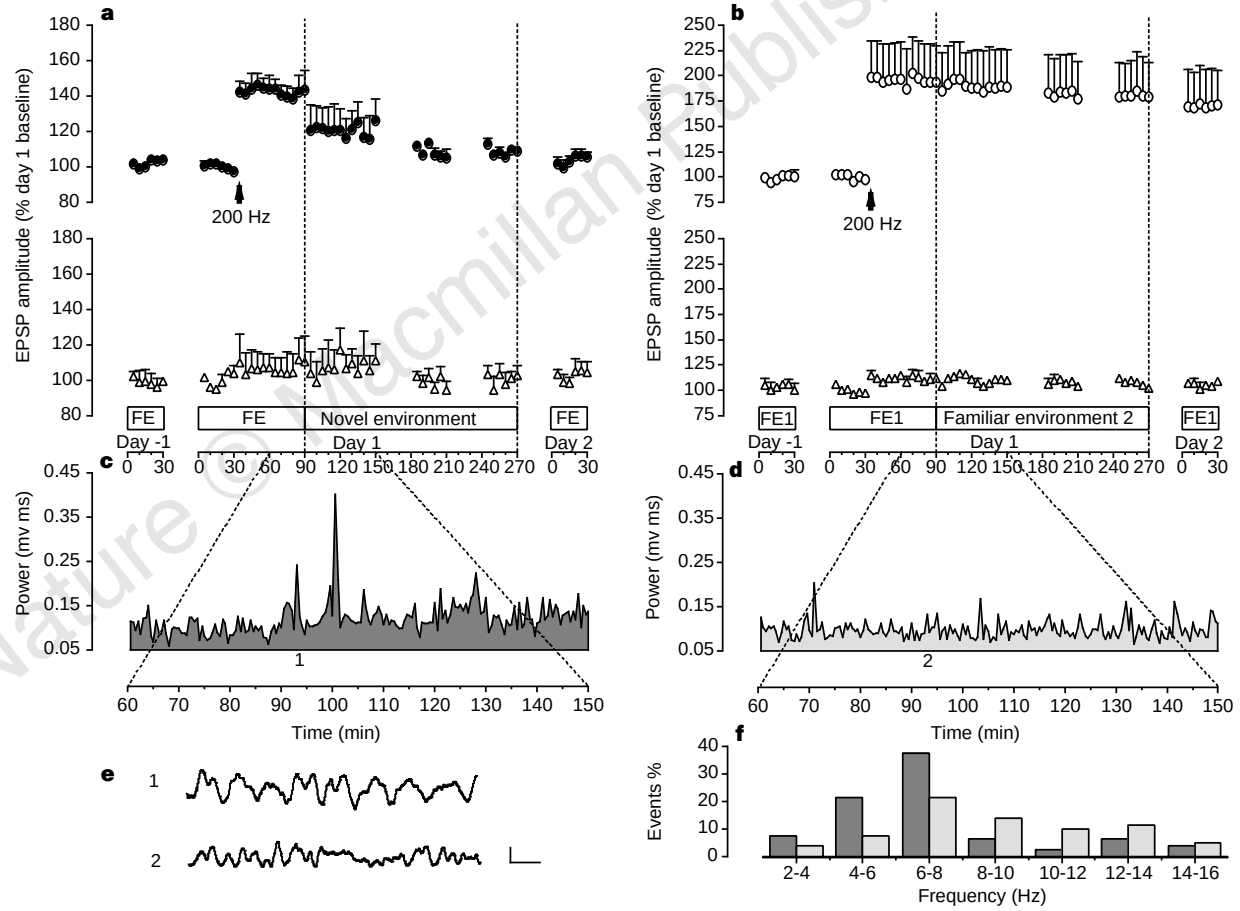

Figure 2 Exploration of a familiar environment fails to affect LTP persistence. **a**, Potentiated responses were reduced in animals that freely left the familiar environment and explored the novel environment for the first time. The test-pathway EPSP decreased from 143.1 ± 11 to 120.4 ± 12.1% at 1 h after entry into the box (black circles, $P < 0.01$ compared to the potentiated level and $P > 0.05$ compared to baseline; $n = 5$) and stayed at baseline for the rest of the experiment (105 ± 2.9% at 24 h). Control pathway responses (white triangles, 107.5 ± 12.4, 107.9 ± 11.1 and 102.6 ± 3.1 at 1, 2 and 24 h after the tetanus in the test pathway) did not change significantly. **b**, There was no change on entry into the box after a 1-h session of familiarization on the two previous days. LTP was induced 1 h before allowing the rat to cross from the familiar box (familiar environment 1, FE1) to the now familiar 'novel' box (familiar environment 2). There was no significant change in the level of potentiation in the test pathway (circles, 193.7 ± 36.1, 188.4 ± 37.7, 178.6 ± 34.1 and 171.2 ± 34.2% at 1, 2, 4 and 24 h after the conditioning stimulation; $P > 0.05$, $n = 5$) or in baseline transmission in the control pathway (triangles, 111.5 ± 3.9, 109.9 ± 3.6, 102.5.6 ± 4.4 and 100.6 ± 6.1% at 1, 2, 4 and 24 h after the conditioning stimulation in the test pathway, $P > 0.05$). **c–f**, First-time, but not third-time, exploration of the box increased the power of the dominant frequency of the hippocampal EEG. **c**, The power increased after the animal explored the box for the first time ($P < 0.01$; 4 animals from **a**). **d**, There was no change after the rat explored the box for the third time ($P > 0.05$; 4 animals from **b**). **e**, Typical traces from **c** and **d**. Horizontal bar, 100 ms; vertical bar, 0.1 mV. **f**, The dominance of 6–8 Hz theta activity increased on first-time exploration (dark bars, $P < 0.05$ compared to third-time exploration, stippled bars, during the first 10-min period after entering the box).

when recordings were taken in the familiar box (Fig. 2a).

We reasoned that if the reversal of LTP was due to experience-dependent hippocampal activation during the exploration of the new box, then re-entry into the same environment after a period of familiarization would not be expected to affect the persistence of recently established LTP. We allowed a group of animals to explore the novel box for a 1-h period on two consecutive days. On the third day, LTP was induced in the familiar box. One hour later, the animals entered and explored the 'novel' box (the second familiar box) for the third time. In these animals, the amplitude of synaptic responses in both the test and control pathways remained stable for the remainder of the experiment (Fig. 2b). The presence of theta electroencephalographic (EEG) activity on entering the novel box was used as an indicator of hippocampal activation[10–12]. Animals that entered the novel box for the first time had greater theta activity, with a dominant frequency of 6–8 Hz, than those that explored the same box for the third time (Fig. 2c–f). As this difference was observed even when the level of exploratory motor activity was matched between the groups (for example, during the first ~5–10-min period of exploration; Fig. 2c–f), the relative increase in 6–8 Hz theta activity is indicative of an altered hippocampal state associated with the processing of new information, rather than just locomotion[10–12]. This is strong evidence that it was the process of assimilation of new information about the novel box, rather than some other aspect (such as entry into a darker area), that was responsible for inducing the reversal of LTP.

We also investigated the time window in which exposure to the novel environment was able to affect LTP. In the experiments already described, the change in environment was introduced 1 h after LTP induction, a time when LTP maintenance is considered to be largely independent of protein synthesis[13]. A further set of experiments examined whether the induction of this 'early' phase of LTP was affected by introduction to a novel environment. When the animals were allowed to enter the novel box 5 min before the application of high-frequency stimulation, the stimulation induced an LTP that was stable for at least 1 h after its induction (Fig. 3); thus, the mechanisms necessary for the induction of early-phase LTP were not blocked by the new situation. Another set of experiments studied the effect of introducing the animals to the novel environment 24 h after the induction of LTP, a time when LTP is dependent on both protein and RNA synthesis[13]. In these animals, exposure to the new environment failed to affect LTP (Fig. 3), indicating that once 'late'-phase LTP has been consolidated, acquisition of information about a new environment had no significant effect on potentiated synaptic responses.

A rapid reversal of LTP ('depotentiation') in the CA1 area of the hippocampus of freely moving adult rats has been reported to be induced by low-frequency stimulation (1–10 Hz) (refs 14–16, but see ref. 17). It is interesting, given the presence of increased theta activity during the induction of naturally occurring reversal of LTP reported here, that the optimal frequencies for artificially inducing depotentiation are in the 5–10-Hz theta frequency range and that stimulation locked to the negative phase of ongoing theta activity is particularly effective[15,18,19]. During exposure to novelty, hippocampal theta activation can occur synchronously across large populations of hippocampal principal neurons and this synchronization appears to gate processing throughout the hippocampal network in a phase-specific manner[11]. There is a growing body of evidence that the detection of novelty is important in hippocampal information storage[20–22]. The time window for the reversal of LTP reported here supports the idea that the hippocampus may hold on to new information until consolidation. Most theories of hippocampal function in memory assume that information is encoded and stored at low density and in a widely distributed manner, thus increasing its storage capacity[23]. The finding of complete reversal of recently induced, large-magnitude LTP during exploration of a new environment indicates that extensive experience-dependent reductions in synaptic strength may occur throughout the hippocampal network. Thus the acquisition of new information via the hippocampus may lead to a widespread and complete depotentiation of most recently potentiated synapses, in tandem with a sparsely distributed potentiation of selected synapses. This may explain the failure of this and previous studies to detect long-lasting increases in synaptic strength at so-called 'naive' pathways in the hippocampus following spatial exploration[4–6]. A redistribution of synaptic efficacy provides a learning mechanism with little net change in synaptic weight[24]. The active erasure of synaptic efficacy changes, within a defined time after they are initiated, provides protection from lasting effects of inconsequential inputs and endows the system with enhanced combinatorial plasticity[25]. From our results, reversal of previously established synaptic strengthening may act as a counterpoint to, and presumably together with, the

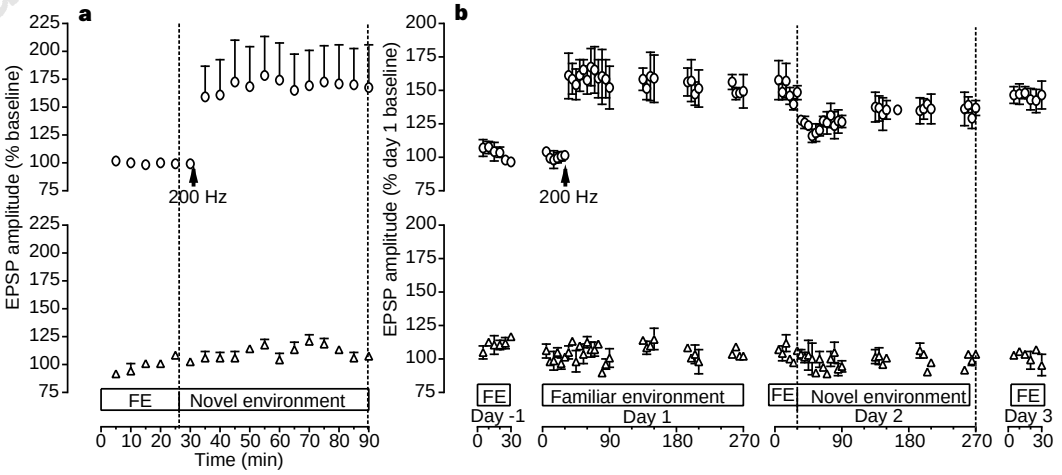

Figure 3 Time window for the effect of novelty exploration on LTP. **a**, LTP induction was not affected by entry into the novel box. When high-frequency (200 Hz, arrow) stimulation was applied to the test pathway 5 min after the animal had entered the novel box, significant LTP was induced (circles, 168 ± 37.8 at 1 h, $P < 0.01$, $n = 5$). Synaptic responses to stimulation of a control input (triangles, 111.7 ± 3.5%, $P > 0.05$) did not change during the experiment. **b**, Well established LTP was not affected by novelty exploration. Stable LTP was induced in the test pathway, which persisted for 24 h after the high-frequency stimulation (200 Hz, arrow). Allowing the animal to enter freely and explore the novel box at this stage had no effect on synaptic responses either in the test pathway (circles; 152 ± 16, 148.3 ± 5.3, 126.7 ± 4.5, 135.8 ± 11.1 and 146.6 ± 10.6% of baseline at 1, 24, 25, 28 and 36 h after the conditioning stimulation, $P > 0.05$, $n = 4$) or the control pathway (triangles; 98.7 ± 4.4, 102.5 ± 2.6, 97.4 ± 5, 100.4 ± 2.7 and 103.2 ± 4.5% at 1, 24, 25, 28 and 36 h after the conditioning stimulation in the test pathway, $P > 0.05$).

strengthening of neuronal connections during the detection and storage of new information by the hippocampus. □

Methods

Electrode implantation and electrophysiology. Experiments were carried out on freely behaving male Wistar rats (200–300 g) that had electrodes implanted under pentobarbitone (60 mg kg^{-1}) anaesthesia. Recordings of field EPSPs were made from the CA1 stratum radiatum of the hippocampus in response to ipsilateral stimulation of the Schaffer collateral/commissural pathway using techniques similar to those described[26,27]. Animals recovered at least 14 days before the start of the experiment. Test EPSPs were evoked at a frequency of 0.033 Hz and at a stimulation intensity adjusted to give an EPSP amplitude of 50% of maximum. The high-frequency stimulation protocol for inducing LTP consisted of 10 trains of 20 stimuli, interstimulus interval 5 ms (200 Hz), intertrain interval, 2 s. Repeated stimulation with this protocol fails to increase the magnitude of LTP, indicating that it is almost at saturation for the group of synapses under observation[26]. LTP was measured as mean ± s.e.m.% of baseline EPSP amplitude recorded over at least a 20-min baseline period. The EEG was simultaneously monitored (from the hippocampal recording electrode) during all experiments so as to ensure that no abnormal activity was evoked by the conditioning stimulation and to monitor hippocampal theta EEG activity. The spectral power of the EEG was measured after fast Fourier transformation of sweeps of 1.2 s duration. Dual pathway experiments, with two independent ipsilateral stimulation inputs to the same recording electrode, were carried out for most experiments. Lack of paired-pulse interaction with responses evoked in the test pathway was used as a criterion of independence.

Recording apparatus and novelty exploration. To allow free exploration without extensive locomotion (which affects brain temperature and field potential measures of synaptic transmission[4,6,28,29], the recording boxes were relatively small (0.07 or 0.08 m^2). Under these conditions, only very transient (<10 min) and small changes (<1 °C) in brain temperature were observed on entering the novel environment.

Experiments were carried out in a well lit (~750 lux, fluorescent lighting) room. The familiar box was made of clear perspex, whereas the novel box was made of Perspex covered with a thin sheet of plastic which acted as a red filter (>600 nm, filter factor ~3×). The boxes in the first study had different shapes (34 × 24 × 24 cm for the familiar, versus 32 × 21 × 20 cm for the novel box). In the other studies, an opaque barrier that separated the familiar and novel environments was removed at 90 min and was closed 20 min later when the animal was in the novel box. To make the novel environment more distinct, the bedding was also different (none in the familiar, versus wood shavings in the novel box). The bedding was changed between rats but was not changed after each trial for a given rat. Behavioural evidence that the animals acquired information about the new environment was provided by the observation that the animals explored less on re-exposure to the novel box on consecutive days (for example, 24 ± 6 versus 14 ± 4 transitions between the familiar and novel boxes in the first 20 min on the first and third day, respectively; $P < 0.05$). Entry into the novel box did not elicit any observable stress responses either hormonally (plasma corticosterone, 5.2 ± 1.2 versus 3 ± 0.8 μg dl^{-1} in familiar box, measured by HPLC; $n = 4$)[27] or behaviourally (no evidence of behavioural freezing, piloerection or defecation typical of stress). The animals were housed individually in their home cage between recording sessions. Statistical comparisons were made by using Friedman two-way analysis of variance by ranks and Mann–Whitney *U*-test where appropriate.

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Decreased lesion formation in CCR2$^{-/-}$ mice reveals a role for chemokines in the initiation of atherosclerosis

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Chemokines are proinflammatory cytokines that function in leukocyte chemoattraction and activation and have recently been shown to block the HIV-1 infection of target cells through interactions with chemokine receptors[1,2]. In addition to their function in viral disease, chemokines have been implicated in the pathogenesis of atherosclerosis. Expression of the CC chemokine monocyte chemoattractant protein-1 (MCP-1) is upregulated in human atherosclerotic plaques[3,4], in arteries of primates on a hypercholesterolaemic diet[5] and in vascular endothelial and smooth muscle cells exposed to minimally modified lipids[5,6]. To determine whether MCP-1 is causally related to the development of atherosclerosis, we generated mice that lack CCR2, the receptor for MCP-1 (ref. 7), and crossed them with apolipoprotein (apo) E-