Defective place cell activity in nociceptin receptor knockout mice with elevated NMDA receptor-dependent long-term potentiation

Franco A. Taverna[1], John Georgiou[1], Robert J. McDonald[2], Nancy S. Hong[2], Alexander Kraev[1], Michael W. Salter[3], Hiroshi Takeshima[4], Robert U. Muller[5,6] and John C. Roder[1]

[1] *Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5*
[2] *Department of Psychology and Neuroscience, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4*
[3] *Department of Physiology, Programme in Brain and Behaviour, Hospital for Sick Children, University of Toronto, Toronto, Canada M5G 1X8*
[4] *Department of Biochemistry, Tohoku University, Graduate School of Medicine 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan*
[5] *Department of Physiology, SUNY-Downstate, Brooklyn, NY 11203, USA*
[6] *MRC Centre for Synaptic Plasticity, Bristol BS8 1TD, UK*

There is growing evidence that NMDA receptor-dependent long-term potentiation (LTP) in the hippocampus mediates the synaptic plasticity that underlies spatial learning and memory. LTP deficiencies correlate well with spatial memory deficits and LTP enhancements may improve spatial memory. In addition, LTP deficiencies are associated with abnormal place cells as expected from the spatial mapping hypothesis of hippocampal function. In contrast, nothing is known on how enhanced NMDA receptor-dependent LTP affects place cells. To address this question we recorded place cells from mice lacking the nociceptin receptor (NOP_1/ORL_1/OP4) that have enhanced hippocampal LTP. We found that the enhanced LTP was mediated by NMDA receptors, did not require L-type calcium channels, and occurred only when high frequency tetanizing stimulus trains were used. Place cells in nociceptin receptor knockout mice were abnormal in several ways: they were less stable, had noisier positional firing patterns, larger firing fields and higher discharge rates inside and outside the firing fields. Our results suggest that excessive LTP can cause subnormal hippocampal place cell function. The effects of LTP enhancement on place cell function may therefore also depend on molecular details of synaptic plasticity, including the relationship between stimulus frequency and synaptic strength, and not merely on the magnitude of synaptic strength increases. The data have important clinical implications on development of strategies to improve cognitive function.

(Resubmitted 6 February 2005; accepted after revision 17 March 2005; first published online 17 March 2005)
Corresponding author F. A. Taverna: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue Toronto, Ontario, Canada M5G 1X5. Email: taverna@mshri.on.ca

Genetically modified mice have been valuable in determining how changes in NMDA receptor-based long-term potentiation (LTP) cause changes of memory function in the whole animal. Specifically, a great deal of evidence indicates that LTP deficiencies at the Schaffer collateral synapse between CA3 and CA1 pyramidal cells cause deficits in the ability to learn or act on spatial information (see Lynch, 2004 for review) as predicted by the cognitive mapping theory of hippocampal function (O'Keefe & Nadel, 1978).

As an additional step to link NMDA receptor-dependent LTP to memory, several studies have recorded place cells from genetically modified mice (McHugh *et al.* 1996; Rotenberg *et al.* 1996, 2000; Cho *et al.* 1998; Yan *et al.* 2002; Nakazawa *et al.* 2003). In the cognitive mapping theory, place cells are taken as the network level units for representing the environment and for computing solutions to spatial problems because they show location-specific firing; they are intensely active only when the animal's head is inside a limited, stable region called the cell's firing field and are otherwise virtually silent. The cognitive mapping theory predicts that alterations of NMDA receptor-based LTP that affect spatial memory also will alter place cell activity. Specifically, genetic manipulations that impair LTP should cause deficits in spatial memory and these deficits should be accounted for by defective place cell properties. An example of this reasoning comes from R(AB) transgenic mice that express an inhibitory

F. A. Taverna and J. Georgiou contributed equally to this work.

 DOI: 10.1113/jphysiol.2004.081802

form of the protein kinase A (PKA) regulatory subunit. Due to low PKA activity, R(AB) mice show labile LTP, transient memory for contextual fear conditioning and unstable place cells (Abel *et al.* 1997; Rotenberg *et al.* 2000). Since these effects have parallel time courses, the implication is that the labile LTP renders place cells unstable and in turn the unreliable map formed from unstable place cells is the origin of the memory defect. Experiments on mice with region-specific knockout of the NMDAR1 gene yield further evidence that LTP underlies key place cell properties and is necessary for normal spatial memory (McHugh *et al.* 1996; Nakazawa *et al.* 2003).

A different way of establishing the role of LTP is to ask how spatial memory performance is affected by *enhanced* LTP. Tang *et al.* (1999) found that forebrain overexpression of the NMDA receptor 2B subunit increases the duration of the open state of the NMDA receptor and is associated with improved memory. However, relationships between LTP properties and spatial learning can be complex and examples exist in which increased LTP produces deficient spatial memory (Silva, 2003). For example, in GluR2 knockout mice successive tetanic stimulation produces a staircase-like sequence of synaptic strength increases rather than the saturation of potentiation seen in normal animals (Jia *et al.* 1996). This enhancement of LTP, however, causes an impairment rather than an improvement in a spatial memory task (Gerlai *et al.* 1998). How can this result be reconciled with the cognitive mapping theory? In this case, there is a simple answer: the LTP in GluR2 knockout mice is NMDA receptor independent, non-Hebbian and non-saturable. With such an abnormal basis for synaptic strengthening, it is not surprising that information storage is compromised. This reasoning leads to the prediction that place cells in GluR2 knockout mice will be defective and in fact they are both noisy and unstable (Yan *et al.* 2002).

To further investigate links between LTP and spatial memory, we recorded place cells in NOP_1/ORL_1/OP4 knockout mice, which we will refer to as nociceptin receptor (NocRKO) knockout mice (Nishi *et al.* 1997; Manabe *et al.* 1998; see review by New & Wong, 2002). This is the first report in which place cells were recorded from mice with enhanced NMDA receptor-dependent LTP. We found that LTP is increased selectively at high, but not low, stimulus frequencies, and an enhanced LTP persists even when L-type calcium channels are blocked. To our surprise, place cells in NocRKO mice were very noisy and their spatial firing patterns were unstable over short (1 h) and long (48 h) intervals between recordings. The results demonstrate that synapses with enhanced NMDA receptor-dependent plasticity can be associated with place cells which have defective firing properties.

Methods

Place cell recordings

Methods to surgically implant electrodes, record place cells and analyse data were similar to those in earlier work (Rotenberg *et al.* 2000; Yan *et al.* 2002). Place cell recordings were made with four tetrodes threaded through a 26 gauge stainless steel cannula (Small Parts Inc., Miami Lakes, FL, USA) so that they all moved together. Each tetrode was a spiral wound bundle of four 25 μm Formvar-insulated nichrome wires (California Fine Wire Co., Grover Beach, CA, USA). The cannula and wires were supported on a moveable Teflon platform through which three 0-80 1/2 inch machine screws were inserted. The screws were tapped into Teflon cuffs that were subsequently fixed to the animal's skull with dental cement. A screw advance of 1/8 turn caused the electrodes to advance 40 μm into the brain. The initial position of the electrode tips was 2 mm lateral to midline, 2 mm posterior to bregma and 800 μm below the dura. Surgery was performed in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, Ottawa, Canada) http://www.ccac.ca/english/publicat/pubframe.htm. The mice were deeply anaesthetized with isofluorane. Temgesic was administered post-operatively for pain relief. Eight NocRKO mice and eight wild-type (WT) litter mates between 4 and 7 months of age were used; these were genotyped by PCR (see below). Data that met criteria for adequate electrophysiological recordings (see below) were collected from five NocRKO and four WT mice.

The signals from eight wires (2 of the tetrodes) at a time were amplified 10 000 times, band-pass filtered at 300–10 000 Hz, digitized at 30 kHz and collected on a computer with Datawave Enhanced Discovery software. Mouse position was tracked by digitizing the location of a light-emitting diode (LED) attached to the electrode carrier on the animal's head. The LED position was found at a time resolution of 60 Hz and a spatial resolution of 6 bits such that each of the 4096 pixels was 1.4 cm on a side. Individual unit waveforms were isolated manually using cluster cutting software from Datawave (Common package or Autocut).

Screening for place cells was performed as mice foraged for fruit loop powder inside a 49-cm diameter, 34-cm high cylindrical apparatus. The apparatus was centred inside a set of cylindrical curtains 2 m in diameter that provided visual isolation of the uncontrolled stimuli in the recording room. The floor of the cylinder was covered with thick black paper that was replaced before each session. The cylinder used for training was grey except for a white card that occupied 90 deg of the cylinder circumference. If no place cells were found, the electrode was lowered by 40 μm, the mice were returned to their home cage and re-screened after several hours or the next day. Once useful place cells

were found (signal to noise > 3, waveform well isolated, presence of complex spikes) the first experimental session 'A' of 16 min was done. This was followed by session B 1 h later after the cylinder and card were rotated 90 deg clockwise. The three subsequent sessions, C, D and E, were all done with the card and cylinder in the original position. The third session C was recorded 4 h after B. Session D was recorded 24 h after session C. The fifth session E was recorded 48 h after session D. Once satisfactory place cells were recorded in session A, each of the subsequent four sessions were done.

Pyramidal cell waveforms were distinguished by their relatively long-duration initial phase (> 300 μs), their tendency to fire high-frequency, decrementing bursts of action potentials (complex spikes; Ranck, 1973) and the occurrence of long intervals (> 1 s) of silence; place cells are a subset of pyramidal cells. Putative inhibitory interneurones (theta cells; Ranck, 1973) were distinguished by their brief initial phase (< 300 μs), lack of complex spikes, increased firing rates during animal movement and a consistently high level of activity (> 5–10 Hz).

Positional firing patterns were analysed by constructing positional firing rate distributions as previously described (Rotenberg *et al.* 2000; Yan *et al.* 2002). Time-averaged firing rates were calculated by dividing the number of spikes in each pixel by the dwell time in that pixel. The rate distributions were visualized using colour-coded firing rate maps. Pixel rates were sorted in ascending order, partitioned into six categories and coded in the ascending sequence yellow (0 Hz), orange, red, green, blue and purple. Unvisited pixels in the cylinder and pixels outside the cylinder were coded white. A place field was defined as a group of contiguous pixels in which each pixel had a firing rate > 0 and shared an edge with another pixel already part of the field (Kubie *et al.* 1990). Coherence was used to estimate the strength of the positional signal from place cells (Kubie *et al.* 1990). Coherence is a nearest neighbour two-dimensional auto-correlation that measures the local smoothness of a positional firing pattern. To calculate coherence, a list of the firing rate in each pixel and a corresponding list of the average firing rate in the eight nearest neighbour pixels was constructed. The reported value is the *z*-transform of the correlation coefficient for these lists.

To measure the stability of place cell positional firing patterns, we calculated a similarity score for each of the four pairs of sessions. The similarity is the *z*-transform of the pixel-by-pixel correlation obtained by superimposing the positional firing pattern in one session against that of the second session (Kubie *et al.* 1990). Maximum similarity scores were also computed by rotating one of the maps in a pair of sessions in 1 deg increments and calculating the correlation coefficient between the two maps at each increment. The maximum similarity score and the angular displacement to obtain that score was recorded.

Genotyping of mice

Mouse genotyping was done with the primer pair nociF (5′-atgggacccgcctacctgaggatgacat) and nociR (5′-gcccatcgaggtgttcatgtgcctgt) which amplified a WT-specific fragment of 378 bp, and with the primer pair nociR (as above) and lacZ (5′-caatatcgcggctcagttcgaggtgc) which produced a 384 bp NocRKO-specific fragment. PCR reactions were run with 20–50 ng of DNA prepared from tail biopsies, using buffer no. 3 of Expand Long Template PCR kit (Roche Diagnostics), Expand polymerase and the following program: 1 × (95°C for 3 min), 5 cycles of 95°C for 15 s, 70°C– 60°C touchdown for 60 s, 72°C for 30 s, followed by 30 cycles of 95°C for 15 s, 60°C for 60 s and 72°C for 30 s.

Electrophysiology in hippocampal slices

Adult male mice were anaesthetized, decapitated and their brains quickly removed into ice-cold artificial cerebral spinal fluid (ACSF) consisting of: 124 mM NaCl, 26 mM $NaHCO_3$, 10 mM D-glucose, 3 mM KCl, 2.0 mM $CaCl_2$, 2.0 mM $MgSO_4$, and 1.3 mM NaH_2PO_4 saturated with 95% O_2–5% CO_2. Using a tissue chopper, 400 μm hippocampal slices were prepared and placed in a holding chamber at room temperature for at least 1 h before use.

Electrophysiological recordings were done in a perfusion-style chamber with ACSF maintained at 30°C with a heat controller and refreshed with a mini-pump. For experiments with D-AP5 or verapamil hydrochloride (Sigma), the drug was added to ACSF from stock solutions (25 mM stock or 50 mM in distilled water, respectively) prepared freshly from powder. Extra care was taken to protect verapamil solutions from light exposure. Glass microelectrodes containing ASCF without Ca^{2+} (3 MΩ resistance) were used to record extracellular field potentials including population excitatory post-synaptic potentials (fEPSPs). Responses were collected every 10 s, amplified and filtered at 2 kHz using an Axopatch 1D, then digitized at 10 kHz using pCLAMP6 software (Axon Instruments). For most experiments, glass recording micropipettes were positioned in the hippocampal CA1 stratum radiatum region to record activity evoked by stimulation (tungsten electrodes) of Schaffer collateral axons. In the experiments in Fig. 7*C* and *D* on WT slices, recording electrodes were placed in the CA1 stratum oriens (subicular side) to record the basal dendritic field potentials evoked by stimulation from the fimbrial side (see Çavus & Teyler, 1998). For stratum radiatum and basal dendrite LTP experiments, stimulus intensity was adjusted to yield fEPSPs that were 40 or 50% of the maximal spike-free size, respectively. The slope of each fEPSP was calculated between 10 and 50% of the peak. To plot

the response magnitude, the slope was normalized to the control slope obtained during the 10 min baseline recording period prior to tetanus; cells lacking stable baseline synaptic responses were not studied. The average baseline fEPSP slope for all 11 sets of LTP experiments (Figs 6 and 7) was -0.46 ± 0.02 mV ms^{-1}, and there were no significant differences for this measure between any of the experimental groups (ANOVA, $P = 1.0$). In the LTP time course plots, each data point reflects an average of six responses (60 s); for clarity, only one data point every third minute is shown. Tetanic stimulation was delivered in the form of four trains (500 ms duration, 10 s apart) at either 20, 50 or 100 Hz. In the basal dendrite recordings we used a tetanus consisting of 20 trains (200 ms duration, 2.5 s apart) at 200 Hz.

Statistical differences were determined by ANOVA or Student's *t* test. LTP comparisons between experimental groups were made by pooling responses for a 10 min window at either 30 ± 5 or 90 ± 5 min post-tetanus. For the D-AP5 experiment, the LTP comparison is a paired *t* test that compared, within animals, baseline responses for the 10 min prior to tetanic stimulation with the responses at 90 ± 5 min thereafter. All error bars reflect standard error of the mean (S.E.M.).

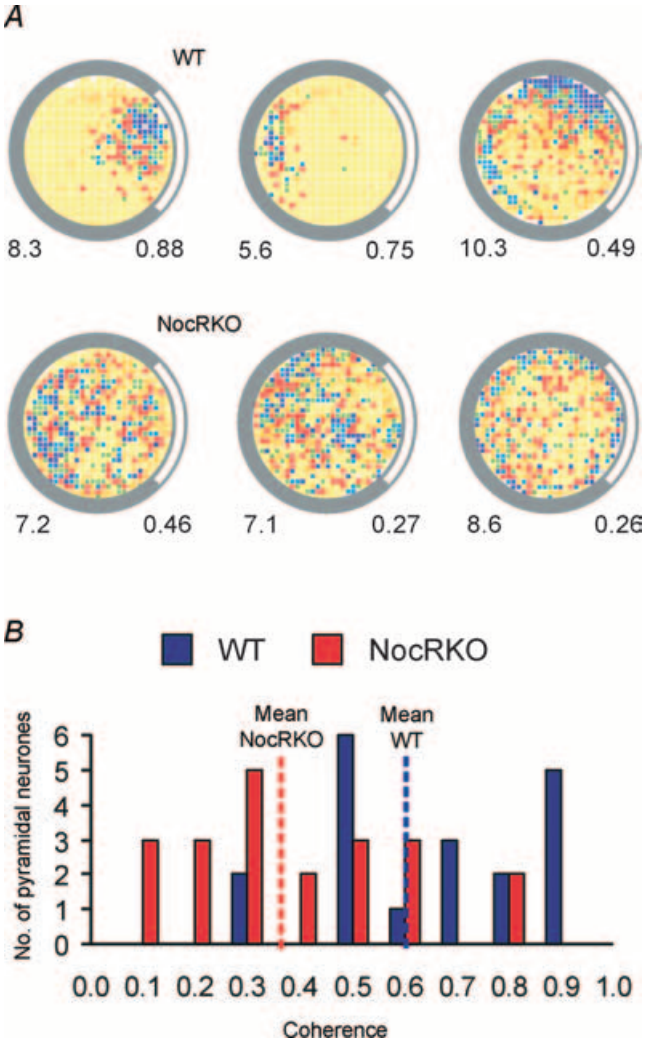

Figure 1. Altered place cell firing fields in NocRKO mice
A, examples of hippocampal CA1 place cell firing fields from WT and NocRKO mice. The circular outline represents the grey cylindrical recording chamber with the white cue card at 3:00 o'clock. Increasing place cell firing rates are sorted in the colour order: yellow, orange, red, green, blue, purple. The firing rate is exactly 0 Hz for yellow pixels. Most NocRKO rate maps were noisier than those of WT mice (as seen by more of the darker pixels and fewer yellow pixels). The median rate in the purple (highest rate) category is given at the bottom left of each map; the corresponding coherence values are listed at the bottom right of each map. *B*, histogram of coherence values of firing fields for 19 WT and 21 NocRKO complex spike pyramidal cells. The mean values for each genotype are shown on the coherence axis.

Results

Place cell firing fields in NocRKO mice are poorly organized

We recorded 39 CA1 pyramidal cells (complex spike cells) from 4 WT mice and 42 from 5 NocRKO mice. After discarding cells with waveform amplitudes $< 150\,\mu$V, we further analysed the discharge of 19 WT and 21 NocRKO neurones. By inspection of colour-coded firing rate maps, the positional discharge patterns for selected WT pyramidal cells were strongly confined to small, well-defined 'firing fields' as shown in the examples of Fig. 1*A*. The spatial discharge of cells from NocRKO mice were more poorly organized as documented by calculating coherence, a measure of the local smoothness of the positional firing pattern. The mean coherence for cells from NocRKO mice was 0.35 ± 0.05 compared with the mean of 0.61 ± 0.05 for cells from WT mice (Fig. 1*B*); the difference in the means was statistically significant (*t* test; T (*t* value) $= 3.88$, d.f. $= 38$, $P < 0.001$).

Pyramidal cells were classified as place cells if their coherence was $= 0.26$; other cells showed either noisy firing patterns or were nearly silent. According to this criterion, of the 19 pyramidal cells in WT mice, 17 were classified as place cells whereas only 14 of the 21 pyramidal cells from NocRKO mice were place cells. Even after this culling procedure, the average coherence of the WT place cells was higher than the NocRKO place cells (0.46 ± 0.05 for NocRKO, 0.65 ± 0.04 for WT; $T = 3.14$, d.f. $= 29$, $P = 0.004$). Thus, deletion of nociceptin receptors significantly reduces the tendency for place cell firing to be tightly confined to a region in space and the firing fields of NocRKO place cells are less precise.

More dispersed and higher rates of firing in NocRKO place cells

The decreased coherence of NocRKO place cells was accompanied by an increase in spike activity. The mean in-field rate for place cells in NocRKO

mice was 4.4 ± 0.7 Hz compared with 2.8 ± 0.3 Hz for WT mice ($T = 2.40$, d.f. $= 29$, $P = 0.02$); the grand average firing rate for NocRKO place cells was 3.8 ± 0.7 H_2 compared with 1.4 ± 0.3 H_2 for WT mice ($T = 3.35$, d.f. $= 29$, $P = 0.002$). The decreased coherence of place cells in the mutant mice therefore cannot be attributed to an increase in apparent noise due to reduced firing sampled over a constant interval. The average fraction of the apparatus area in which place cells fired was 0.77 ± 0.06 for NocRKO place cells *versus* 0.55 ± 0.05 for WT place cells ($T = 2.81$, d.f. $= 29$, $P = 0.009$). Therefore, the positional discharge of NocRKO place cells was more dispersed as well as noisier.

Instability of place cells in NocRKO mice

The positional firing patterns of place cells in NocRKO mice were much less stable than those in WT mice. Examples of sequential recordings for WT and NocRKO place cells are shown in Fig. 2. Note that for the 1 h time point (session B) the cue card was rotated 90 deg clockwise; however, the firing pattern diagrams have been rotated back 90 deg counter-clockwise for ease of visual comparison. Also included are signals from tetrodes to show that the recorded waveforms were stable.

For each of the three place cells from WT mice the positional firing pattern is similar across all five time points (Fig. 2, top). In contrast, the reproducibility of the firing patterns for the three place cells from NocRKO mice is much weaker (Fig. 2, bottom) although fields are preserved in some cases (e.g. the second, third and fifth sessions for NocRKO cell 1). The lower stability of the NocRKO place cells is documented in Fig. 3*A* where for each interval the similarity score calculated for 0 deg rotation (relative to the cue card) is reliably higher at each interval in place cells from WT compared with NocRKO mice.

We also tested the possibility that NocRKO place cells are stable except for arbitrary rotations between sessions, as might occur if the head direction cell system reset to an arbitrary value whenever the mouse was placed in the recording chamber. To this end, we calculated the maximum similarity (Fig. 3*B*) and the angular rotation required to produce the maximum similarity (Fig. 3*C*). As expected, the maximum similarity is higher than the 0 deg similarity for both mouse genotypes at all intervals. Nevertheless, a difference between the genotypes was preserved at all intervals, where again the trend was for higher stability in place cells from WT mice. The tendency of the maximum similarity to increase more for NocRKO mice suggests there may be some merit to the idea that the instability is due in part to reduced constancy of the angle between the cue card and the firing field. This notion receives support from an analysis of the angle required to produce maximum similarity which is much greater for place cells in NocRKO than in WT mice, as shown in Fig. 3*C* for 3 of the 4 intervals and a trend in the same direction for the final interval.

In several earlier studies, rotating the white wall card when the animal was outside the recording cylinder caused equal rotations of place cell firing fields, demonstrating the strong salience of this cue. We tested the extent of cue card control by rotating it 90 deg clockwise during the second session at $t = 1$ h and rotating it back to its initial position during the third session at $t = 5$ h.

The ability of the cue card to control the angular position of the firing field is evident for the example WT place cells in Fig. 2 (1 h interval) where the fields remained in the same position relative to the cue card. In contrast, many of the fields in the NocRKO mice did not rotate in tandem with the cue card. To numerically estimate field rotations caused by 90 deg card rotations we computed the maximum firing pattern similarity between the 0 h and 1 h sessions. The plot of Fig. 3*D* shows that rotations for place cells in WT mice are quite tightly clustered around the expected value of 90 deg whereas the rotations for place cells in NocRKO mice are more broadly dispersed. Formally, however, a Rayleigh test (Batschelet, 1981) indicates that the angular distribution of firing pattern rotations is not random for either NocRKO (vector length $= 0.635$, $n = 12$) or WT mice (vector length $= 0.733$, $n = 9$). Similarly, the V test (Batschelet, 1981) does not reject the hypothesis that the rotation angle is 90 deg for either genotype ($V = 0.576$ for NocRKO mice, 0.731 for WT mice). Nevertheless, the variance deviations of firing pattern rotations away from the expected value of 90 deg is significantly different between NocRKO and WT mice according to an F test ($F = 18.3$, d.f. $= 19$, $P < 0.001$).

The striking across-session instability of positional firing patterns for place cells from NocRKO mice led us to ask if they also were less stable within sessions. Accordingly, we computed the similarity between the initial and final 6 min of the first session for each cell; the middle 4 min were excluded to enhance possible drift of positional firing pattern. The difference between the mean within session similarity of 0.355 for WT place cells and 0.124 for NocRKO place cells was highly reliable ($T = 3.85$, d.f. $= 31$, $P < 0.001$), indicating that the NocRKO cells were unstable even when the mouse was continuously in the recording chamber.

Hippocampal slice electrophysiology

Given our unexpected finding of defective place cells from NocRKO mice, we were interested in characterizing LTP properties in more detail. In previous hippocampal slice work on NocRKO mice, recordings were made in the presence of picrotoxin to block $GABA_A$-mediated transmission (Manabe *et al.* 1998). To evaluate synaptic properties in conditions closer to the *in vivo* place cell recordings, we chose not to use picrotoxin.

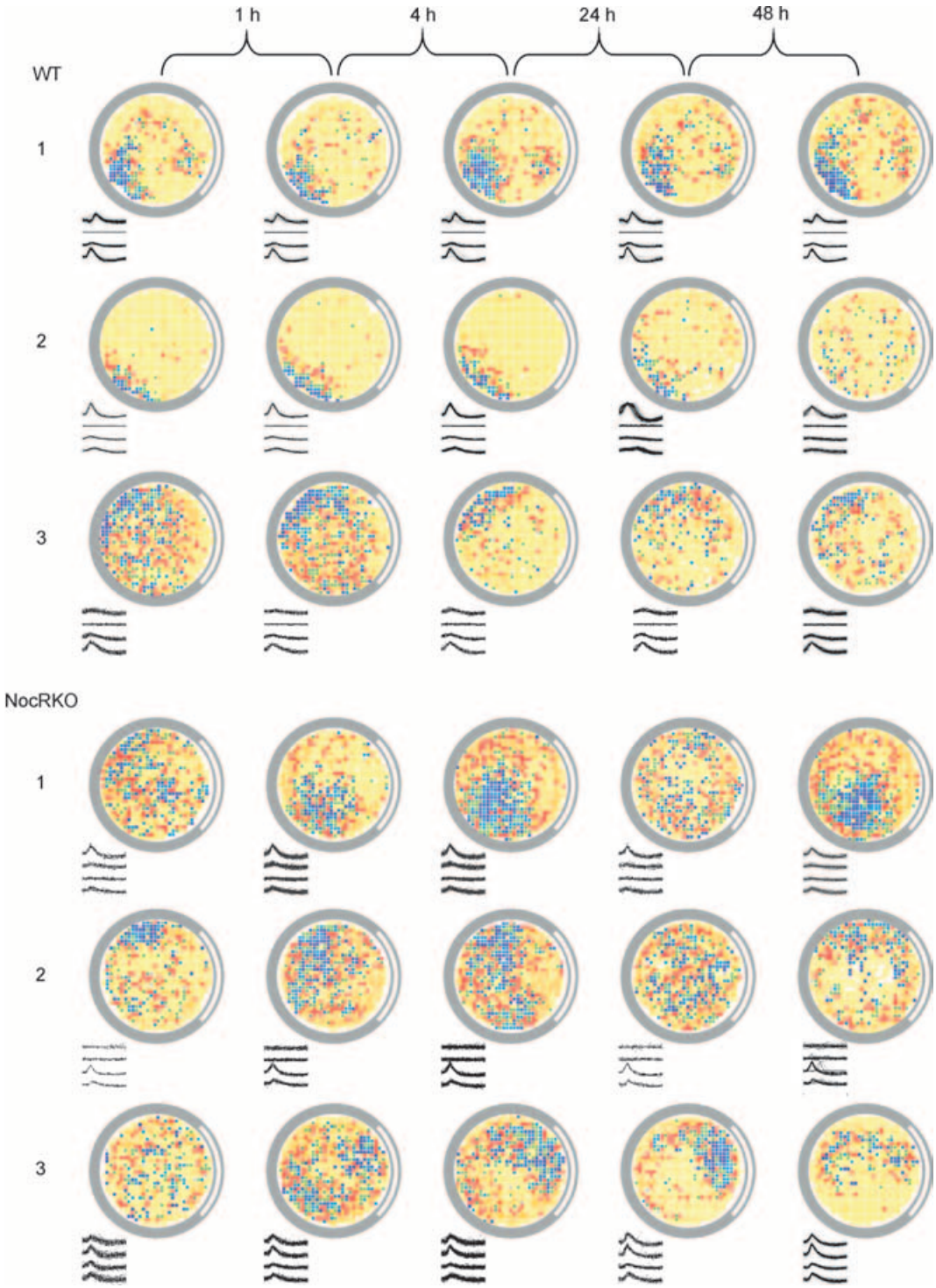

Figure 2. Poor stability of place cell firing fields in NocRKO mice
Representative series of 5 sessions from 3 WT (top) and 3 NocRKO (bottom) place cells. Note that in session B (1 h interval) the cue card was rotated 90 deg clockwise; however, the map shown has been rotated counter-clockwise 90 deg for simplicity of visual comparison. Each row shows repeated recordings from the same place cell with the time intervals indicated above. Tetrode waveforms for each session are displayed below the firing rate maps. The consistency of waveforms demonstrates the temporal stability of the recordings.

We first asked if lack of nociceptin receptors affects basal synaptic transmission at CA3–CA1 synapses in stratum radiatum. CA1 pyramidal neurones were activated by Schaffer collateral stimulation. The magnitude of pre- to post-synaptic neuronal coupling was measured using single pulse stimulation to evoke field excitatory post-synaptic potentials (fEPSPs). When fEPSP slope was plotted against the size of the presynaptic fibre volley, no differences were seen between slices from NocRKO mice and their WT littermates (Fig. 4; ANOVA, $P > 0.05$) indicating no changes in basal synaptic transmission.

To further compare synaptic properties in WT and NocRKO slices we measured paired-pulse facilitation (PPF, a presynaptic form of short-term plasticity) in which CA1 fEPSPs are enhanced if a second stimulus is delivered less than 1 s after a first stimulus. When PPF was plotted as a function of interstimulus interval (Fig. 5) there were no differences between WT and NocRKO mice (ANOVA,

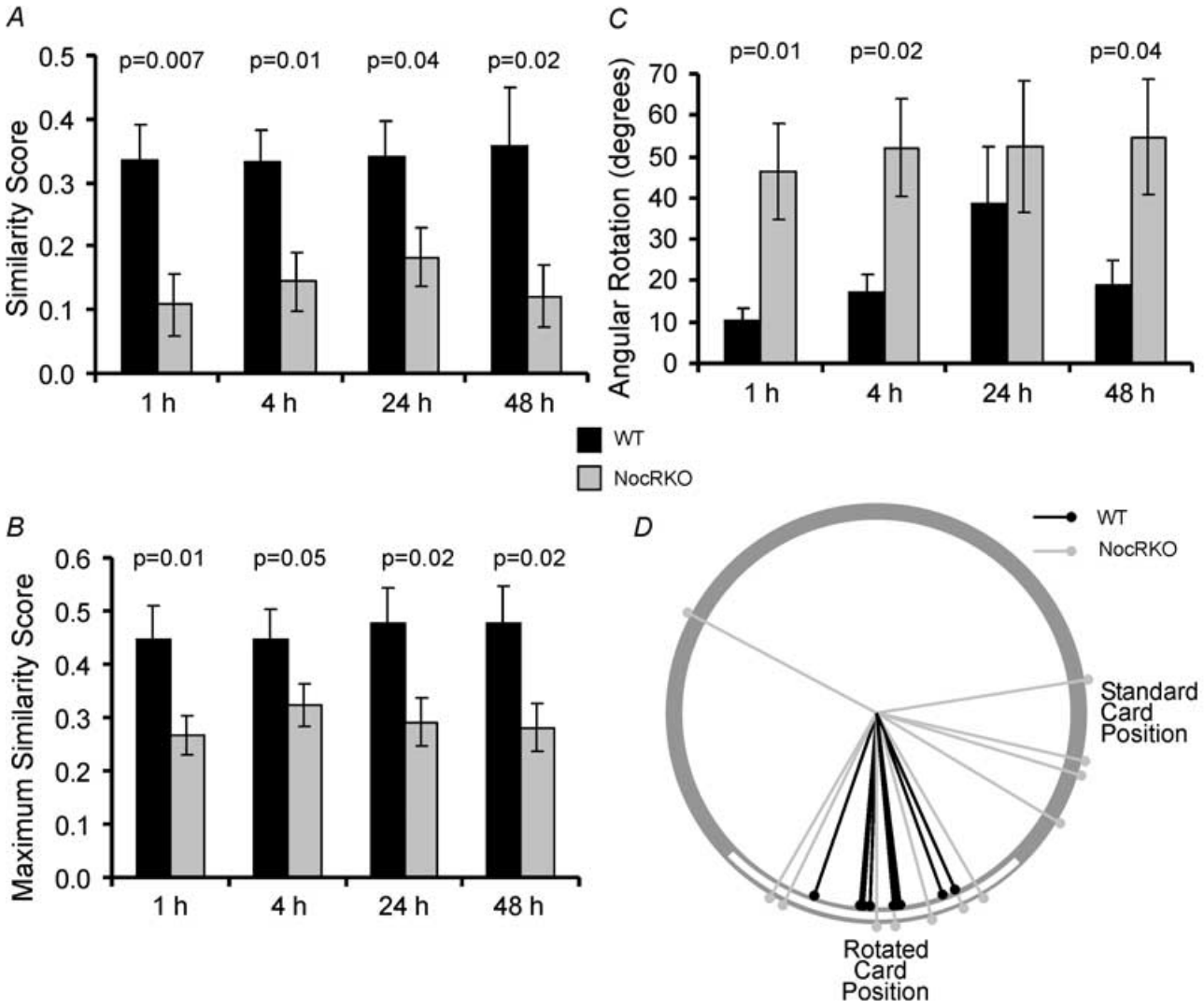

Figure 3. Inaccurate positional pattern of place cell firing fields in NocRKO mice
A, similarity scores for pairs of recording sessions separated by different intervals. Similarity was calculated only at 0 deg (i.e. no optimization) with the cue card rotation for session B subtracted. The similarity for WT firing fields was higher than for NocRKO cells at all intervals indicating greater stability (statistical differences by *t* test are shown). *B*, maximum similarity scores for pairs of recording sessions separated by different intervals. Maximum similarity was found by rotating the second session of a pair against the first in 1 deg steps; the cue card rotation for session B was subtracted. The WT firing fields were more stable than the NocRKO fields according to this measure. *C*, the angle at which maximum similarity occurred was also compared. The smaller rotation required to maximize similarity once more indicates the greater stability of WT firing fields. *D*, polar plot of the rotations required to maximize similarity after cue card rotation. For both genotypes, the rotations systematically follow the card rotation. Nevertheless, the instances are much more concentrated near 90 deg for WT place cells than for NocRKO place cells.

$P > 0.05$ at all intervals shown). Paired-pulse facilitation profiles for slices from WT and NocRKO mice virtually superimpose. We conclude that absence of the nociceptin receptors does not affect basic synaptic properties or PPF and these properties cannot account for the place cell abnormalities in NocRKO mice.

Enhanced Schaffer collateral–CA1 LTP in NocRKO slices

We next proceeded to evaluate LTP of synaptic responses, a well accepted cellular correlate for spatial learning and memory in the hippocampus. After establishing stable baseline responses in stratum radiatum for at least 20 min, a 100 Hz tetanus was given to induce LTP (Fig. 6). Compared with WT slices, NocRKO slices showed a statistically greater amount of synaptic potentiation beginning immediately after the tetanus and persisting even 90 min thereafter (151 ± 8 *versus* $202 \pm 26\%$, respectively, $P = 0.01$). Moreover, using NocRKO slices we performed experiments in which the NMDA receptor blocker D-AP5 was added to the ACSF, and found there was a nearly complete block of LTP induction (Fig. 6*B*); synaptic responses at 90 min after tetanic stimulation were not significantly different from pre-tetanus baseline values ($99 \pm 11\%$; $P = 0.96$). Thus, even in the absence of GABA receptor-mediated inhibition, NocRKO hippocampal slices show supernormal tetanic stimulation-induced LTP that is NMDA-receptor dependent.

The sliding threshold function of plasticity modelled by Bienenstock *et al.* (1982) suggests that synapses can be modified to favour induction of LTP. Indeed, studies on transgenic mice have revealed that the frequency function of LTP can be modified (Migaud *et al.* 1998; Tang *et al.* 1999; Krucker *et al.* 2002; Meng *et al.* 2002). We thus wondered whether LTP induced by lower stimulation frequencies was different in NocRKO mice. Stimulation at 50 Hz produced robust enhancement of the fEPSP for both WT and NocRKO slices but the enhancement was nearly the same (Fig. 6*C*). Additional experiments with a tetanus train frequency of 20 Hz weakly potentiated synaptic responses in slices from both mouse genotypes (Fig. 6*D*). LTP as a function of stimulus frequency is summarized in Fig. 6*E* which shows that at high, but not low, stimulation rates, synaptic potentiation is greater in NocRKO compared with control slices.

Enhanced LTP in NocRKO independent of L-type calcium channels

The enhanced LTP in NocRKO hippocampal slices was limited to high frequency stimulation, suggesting that

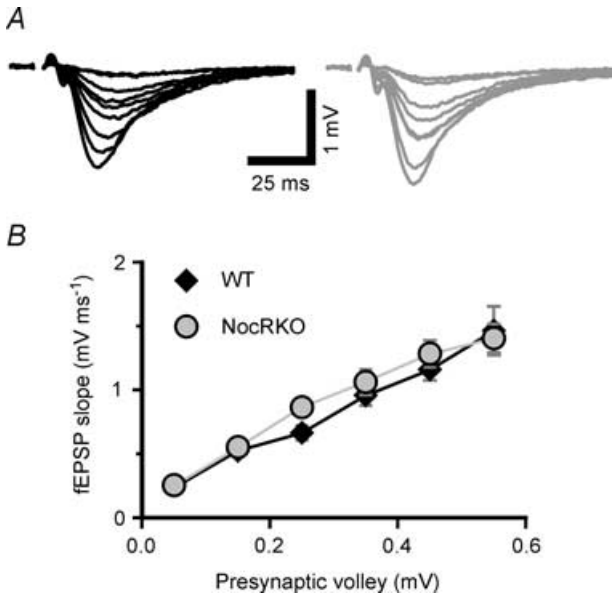

Figure 4. Synaptic transmission in hippocampal slices
A, representative field excitatory postsynaptic potentials (fEPSPs, shown superimposed) were recorded from CA1 stratum radiatum region in response to increasing strength of Schaffer collateral stimulation. WT traces are shown in black (left) and NocRKO traces appear in grey (right). *B*, the slope of the evoked fEPSP was plotted as a function of the presynaptic volley amplitude for both WT (black diamonds) and NocRKO (grey circles) littermates ($n = 8$ and 10 slices, respectively, each with 8 fixed stimulation intensity levels per slice).

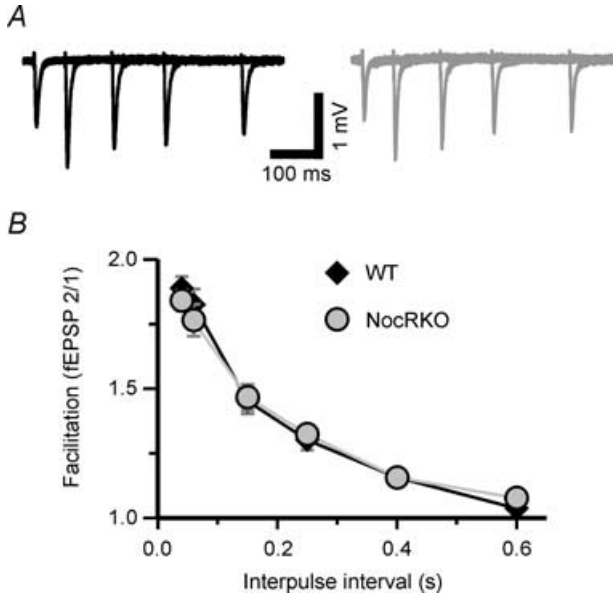

Figure 5. Paired-pulse facilitation is unchanged in NocRKO mice
A, representative responses for WT (black traces, left) and NocRKO (grey traces, right) are shown. Paired stimuli delivered at intervals of 60, 150, 250 and 400 ms are shown in superimposed format. *B*, the facilitation ratio (slope of second fEPSP divided by slope of first fEPSP) was plotted for WT ($n = 8$) and NocRKO ($n = 10$) slices. Paired-pulse facilitation was measured with the stimulus intensity set to make the first fEPSP slope 40% of maximum spike-free value. Note that the two curves are virtually the same.

L-type voltage-dependent calcium channels (VDCCs) may be involved (Grover & Teyler, 1990). Although induction of VDCC-LTP typically requires very high frequency stimulation (200 Hz), 100 Hz tetani can also induce a compound LTP comprised of both NMDA-LTP and VDCC-LTP (Grover & Teyler, 1990; Çavus & Teyler, 1996); the contribution of the latter depends on particular ionic conditions and stimulation parameters (intensity, duration, train pattern). Even though the NMDA receptor blocker D-AP5 blocked LTP induction in NocRKOs, it is possible for VDCCs to participate in LTP when NMDA receptors are not blocked. To test this hypothesis, we performed LTP experiments in ACSF containing the L-type VDCC blocker verapamil. In both NocRKO and WT hippocampal slices, addition of verapamil did not alter stratum radiatum baseline synaptic responses. However, LTP was significantly greater in the NocRKO compared with the WT slices (Fig. 7*A* and *B*), and the enhancement persisted even 90 min after the tetanus (185 ± 16 *versus* $148 \pm 6\%$, respectively, $P = 0.03$).

It was important to have a positive control showing that verapamil could block L-type VDCCs. Synapses at rat CA1 basal dendrites (stratum oriens) are known to express a non-NMDA receptor-dependent type of LTP upon 200 Hz stimulation (sharp-wave-associated CA3 bursts) which can be blocked by L-type VDCC antagonists such as verapamil (Çavus & Teyler, 1998). We recorded synaptic responses from basal dendrites in WT slices, and found that 200 Hz stimulation induced a potentiation that continued to strengthen during the first 10 min and persisted for 60 min (Fig. 7*C* and *D*). In separate experiments on WT slices, addition of verapamil did not affect the basal dendrite baseline fEPSPs; however, verapamil effectively blocked LTP maintenance; the 200 Hz tetanus induced a brief potentiation that returned to near-baseline (pre-tetanus) levels within 30 min ($122 \pm 4\%$; significantly different than vehicle control value of $190 \pm 11\%$, $P = 0.004$). Thus, verapamil can block L-type VDCC-mediated LTP at basal dendrites. This suggests that verapamil should also have blocked such

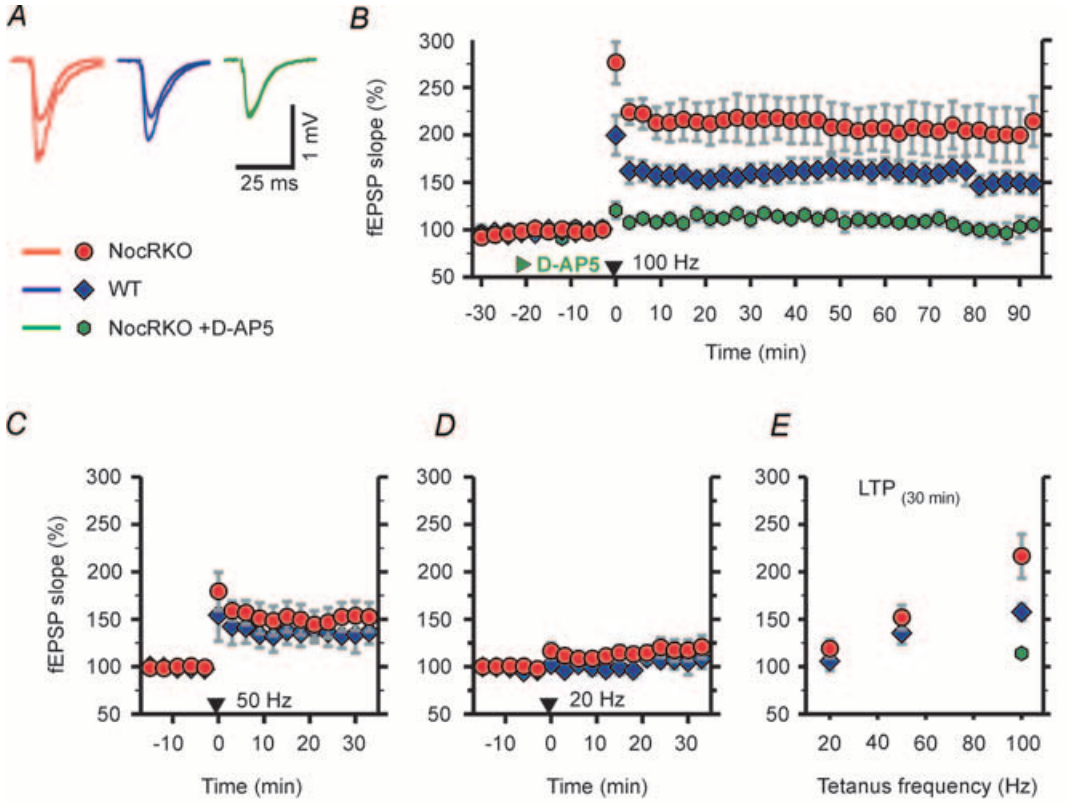

Figure 6. Enhanced NMDA receptor-dependent LTP in slices from NocRKO mice
A, sample fEPSPs recorded before and 90 min after (superimposed) 100 Hz tetanic stimulation to induce LTP at Schaffer collateral–CA1 synapses. From left to right: NocRKO, WT and NocRKO in the presence of NMDA receptor blocker D-AP5. *B*, LTP time course showing ensemble averages of the normalized fEPSP slope (per cent of average pre-tetanus fEPSP slope) for each experimental group (NocRKO, $n = 6$; WT, $n = 6$; NocRKO in D-AP5, $n = 4$). Each data point represents 6 responses averaged over 1 min, and for clarity only every third time point is shown. Time of tetanus delivery is shown, as well as time of drug addition for NocRKO slice group receiving D-AP5. *C*, LTP induced by 50 Hz stimulation ($n = 6$). *D*, induction of LTP by 20 Hz stimulation ($n = 5$). *E*, magnitude of LTP at 30 min induced by different tetanus frequencies. The LTP in NocRKO slices is significantly greater than that of WT slices at 100 Hz.

channels in stratum radiatum, and that the enhanced LTP recorded in NocRKOs does not require L-type VDCCs.

Discussion

According to the spatial mapping theory of hippocampal function (O'Keefe & Nadel, 1978), spatial learning and memory depend on proper activity of place cells and related cells in the neural navigational system. Moreover, NMDA receptor-dependent LTP plays an essential role in consolidating new place cell activity; NMDA receptors must be active during initial exposure to a novel environment for environment-specific firing patterns to be stable over intervals of 24 h (Kentros *et al.* 1998). The development of methods to enhance cognitive function may require improvements in NMDA receptor-mediated signalling. There is no information on how enhanced NMDA receptor-mediated LTP affects place cell function. Thus, to understand relationships between enhanced LTP and place cell properties, we studied place cell activity in mutant mice that do not express the $NOP_1/ORL_1/OP4$ nociceptin receptor.

Electrophysiological effects of nociceptin receptor knockout

Given the unexpected finding of poor place cell function, we speculated initially that the electrophysiological conditions used in Manabe *et al.* (1998) to record LTP were not representative of the conditions we used here to study place cell function. In particular, we were interested in whether the block of GABA receptors or perhaps the age of the animals were important. Thus, we performed electrophysiological experiments to characterize synaptic properties in conditions closer to those of the *in vivo* place cell recordings: (1) we did not block $GABA_A$ conductance; (2) we used a perfusing solution containing a 1 : 1 ratio of Ca^{2+} to Mg^{2+} (2 mM each) since the concentration in the brain is around 1.0–1.5 mM for each (Somjen, 2002); (3) we set the temperature to 30°C rather than 25–28°C; and (4) we used mice that were the same age as those used for place cell recordings (4–7 months old rather than 5–9 weeks). We found that input–output characteristics and paired-pulse facilitation of synaptic responses in stratum radiatum were normal in slices from NocRKO mice. Moreover, there was enhanced LTP in slices from NocRKO slices compared with WT slices after 100 Hz tetani.

Is the enhanced LTP in NocRKO mice mediated by NMDA receptors? It has been shown previously that when $GABA_A$ and NMDA receptors are blocked, post-tetanic potentiation in WT and NocRKO slices is equally enhanced and decays back to baseline within 10 min (Manabe *et al.* 1998). Such findings suggest that (1) this form of presynaptic plasticity is unchanged in NocRKOs, and (2) there is no induction of non-NMDA receptor-dependent LTP in NocRKOs under the above conditions. Regardless,

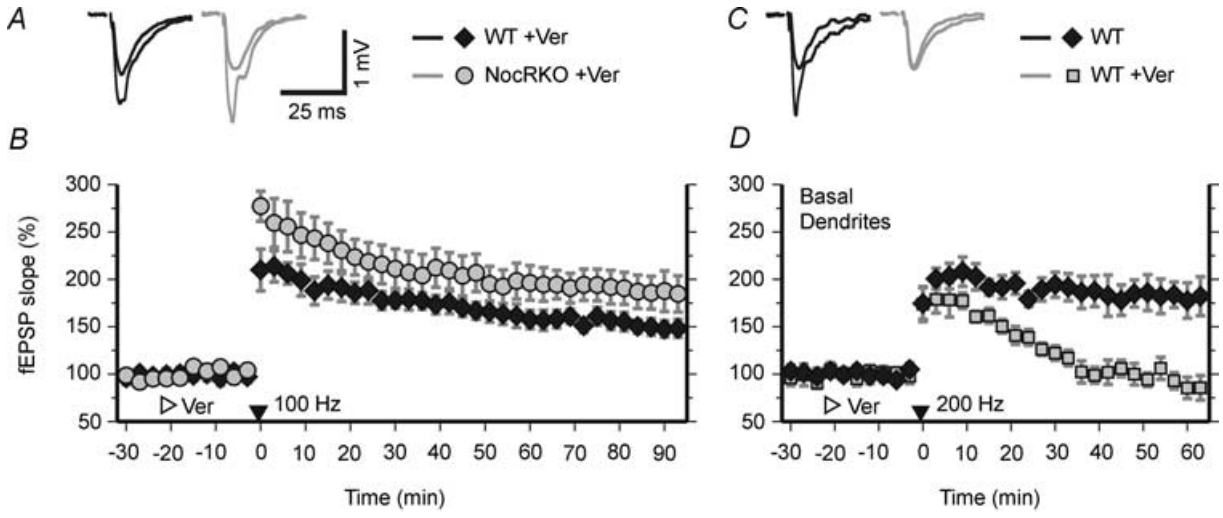

Figure 7. Superior LTP in NocRKO slices independent of L-type calcium channels
A, example fEPSPs recorded in the presence of verapamil (Ver), before and 90 min after 100 Hz tetanic stimulation (traces shown superimposed) to induce LTP at Schaffer collateral–CA1 stratum radiatum synapses. *B*, LTP time course showing group averages of the normalized fEPSP. Time of drug addition and time of tetanus delivery are shown (NocRKO, $n = 8$; WT, $n = 6$). *C*, sample fEPSPs recorded from CA1 basal dendrites (stratum oriens), before and 60 min after 200 Hz tetanic stimulation (traces shown superimposed) to induce L-type calcium channel-dependent LTP. Traces on the left are from WT slices and traces on the right are from WT slices recorded in the presence of verapamil. Scale as per *A*. *D*, basal dendrite LTP time course in WT slices induced by 200 Hz stimulation either in the presence of verapamil ($n = 4$) or vehicle ($n = 4$).

to examine the role of NMDA receptors in LTP under our current recording conditions and tetanus protocol, we tetanized slices from NocRKO mice in the presence of the NMDA receptor blocker D-AP5, and found that LTP induction was almost completely blocked. Thus, NMDA receptors are necessary for LTP induction in NocRKO slices.

There are several transgenic mouse lines that show changes in the frequency function for LTP (Migaud *et al.* 1998; Tang *et al.* 1999; Krucker *et al.* 2002; Meng *et al.* 2002). To examine whether synapses in NocRKOs are modified to favour LTP induction, we performed experiments using 50 and 20 Hz stimulation. In response to these lower frequency tetani, the enhancement in synaptic strength was not statistically different between the genotypes. We conclude that only high frequency tetani can reliably evoke supernormal enhancements in synaptic strength in NocRKO mice.

VDCCs can be activated during strong depolarization, and in particular L-type VDCCs can contribute to LTP induced by 100–200 Hz tetani (Grover & Teyler, 1990; Çavus & Teyler, 1996). This warranted an examination of whether L-type VDCCs contribute to the enhanced LTP recorded in the NocRKO slices. However, in the presence of the L-type antagonist verapamil, we again detected a statistically greater LTP in NocRKO slices compared with WT slices. We confirmed that verapamil was active by performing separate experiments on basal dendrites from WT mice, where we found that LTP was blocked by verapamil; we tested verapamil at basal dendrite synapses because in rat slices, they express a non-NMDA receptor-dependent form of LTP upon 200 Hz stimulation which can be blocked by verapamil (Çavus & Teyler, 1998). Thus, we conclude that L-type VDCCs are not required for the enhanced LTP recorded in stratum radiatum of NocRKO slices.

Mechanisms of enhanced LTP in NocRKO mice

Based on known nociceptin receptor interactions and signalling pathways, the mechanism of enhanced LTP in NocRKO may relate to changes in potassium channel function and/or cyclic AMP signalling (see review by New & Wong, 2002). Nociceptin acts on its receptor to activate a potassium conductance (Madamba *et al.* 1999) that is not present in the NocRKO mice. This conductance acts to decrease cell excitability and its absence therefore provides a plausible explanation of the increased firing rate of NocRKO pyramidal cells. Since potassium channel activation is probably more important during high frequency activation when neuronal depolarization level is high, it is interesting to entertain the possibility that the high frequency dependence of enhanced LTP is a direct consequence of the missing potassium conductance. In this view, lower frequency tetani produce LTP of equal magnitude because the nociceptin receptor-induced potassium conductance plays no role in either WT or mutant mice; in WT mice, each successive stimulus arrives too late for the induced potassium conductance to affect firing level whereas in NocRKO mice the conductance is simply missing. In contrast, 100 Hz tetani in WT mice produce less postsynaptic depolarization since each successive stimulus arrives while the nociceptin receptor-induced potassium conductance is still active from the previous stimulus of the train. Since this influence is missing in NocRKO mice, the magnitude of post-synaptic depolarization and therefore of consequent LTP is increased in a frequency-dependent fashion. The effects of increased LTP may further contribute to the increased firing rate of place cells in NocRKO mice.

An explanation of this kind was suggested and then rejected by Manabe *et al.* (1998) based on the inability to detect differences in depolarization (measured 200 ms into 100 Hz tetanus). The form of extracellular-evoked potentials, however, is not well suited for detecting differences in the trajectory caused by a subtle change in potassium conductance. We therefore regard as an open question the possibility that there might be a single major phenotypic consequence of the knockout, the loss of a potassium conductance, that has an important secondary consequence, the enhanced NMDA receptor-dependent LTP at high stimulus frequency. Alternatively, Manabe *et al.* (1998) proposed that changes in cyclic AMP levels or alterations of protein kinase C or MAP kinase function may underlie the elevated LTP in NocRKO mice. Removal of tonic inhibition of adenylate cyclase activity (via knockout or antisense oligonucleotide-mediated depletion of $G_{i\alpha 1}$) results in enhanced hippocampal LTP (Pineda *et al.* 2004); lack of nociceptin receptor-mediated inhibition of G_i may similarly promote cyclic AMP-mediated signalling and consequently LTP. Recently, Mamiya *et al.* (2003) described a hyperfunction of NMDA receptors in NocRKOs, and also found reduced polysialyated forms of neural cell adhesion molecule. Thus, there are also several alternative mechanisms by which lack of nociceptin receptors might lead to up-regulation of LTP.

Place cell changes in NocRKO mice and implications for spatial behaviour

Place cells in NocRKO mice differ from place cells in WT mice in at least four ways: (1) their firing rates are higher; (2) as measured by coherence, their positional firing patterns are more poorly organized. Reduced coherence may in part be a consequence of position-independent increases of firing rate; (3) their positional firing patterns are unstable within sessions and over a wide range of intersession intervals; (4) their firing fields are poorly controlled by the white cue card, a prominent visual stimulus. These major differences make NocRKO place

cells inferior versions of place cells in WT mice. Indeed, the spatial signal in NocRKO pyramidal cells is often so weak that it is hard to think of them as true place cells.

In the face of these serious defects in spatial firing, it is surprising that in the Morris swimming task, NocRKO mice showed an *enhanced* rate of learning, without any difference in long-term retention of memory (Manabe *et al.* 1998). This behavioural outcome is in disagreement with the spatial mapping theory of hippocampal function (O'Keefe & Nadel, 1978) Several possibilities could explain this discrepancy. (1) The swimming task, as administered by Manabe *et al.* (1998) may not be a proper test of spatial learning and memory. The use of a small (1.2 m) diameter pool may lower or remove the need for navigation based on distal landmarks. More importantly, the very low (18°C) temperature of the swimming pool could have negatively affected WT mice. A problem of this type is suggested by the very long (> 25 s) swimming time to the platform shown by WT mice, a value more than twice as great as we have found in other circumstances. Taking 10 s as a more characteristic latency for WT mice, the NocRKO mice in the Manabe *et al.* (1998) study would show a swimming task impairment, not an improvement. (2) Nociceptin may have unexpectedly complex effects on water maze performance. Thus, although a high nociceptin dose (3.3 nmol infused into rat lateral ventricle) lengthens swimming time, as expected from the reported results in NocRKO mice, doses of 1.0 and 0.33 nmol rat^{-1} resulted in shortened swimming times (Sandin *et al.* 2004). Although the species difference makes comparison less than optimal, the same dose of 1.0 nmol that improved water maze performance in rats impaired retention of contextual fear conditioning in mice (Mamiya *et al.* 2003). (3) The abnormal place cell firing activity may encode enough spatial information to allow adequate or even improved navigational performance. This hardly seems likely since several other genetically altered mice with modifications of LTP properties also resulted in abnormal place cells and deficiencies in spatial performance (McHugh *et al.* 1996; Rotenberg *et al.* 1996; Cho *et al.* 1998; Yan *et al.* 2002).

Are there any differences in place cell firing properties in mice that have enhanced *versus* impaired LTP? A survey of genetically engineered mice in which place cells have been recorded reveals that there is no apparent relationship between direction of LTP and place cell properties. For example, coherence and stability are reduced in mice with enhanced (Yan *et al.* 2002; and herein) or reduced LTP (McHugh *et al.* 1996; Rotenberg *et al.* 1996; Cho *et al.* 1998; Rotenberg *et al.* 2000).

Summary

We find that even in the presence of $GABA_A$ receptor-mediated currents, there is enhanced LTP in NocRKO (compared with WT) mice after 100 Hz tetani. We also find that this elevation does not occur following tetani of lower frequencies. LTP in NocRKO mice, including the elevated component, is blocked by application of D-AP5 and is therefore dependent on activation of NMDA channels. Blockade of L-type VDCCs failed to block the LTP enhancement recorded in NocRKO slices. Despite the ability to express superior LTP, hippocampal pyramidal cells in NocRKO mice show very little of the crisp, stable location-specific firing characteristic of place cells.

Just why an enhancement of LTP leads to a gross defect of spatially tuned firing is unclear, but our results provide evidence that simply boosting the magnitude of plastic changes is not sufficient to improve functioning at the network level. In short, LTP magnitude, even when it is NMDA receptor-dependent, is not correlated in any simple way with place cell function, the putative substrate for complex spatial behaviour in rodents. Our findings motivate a new look at place cell function and spatial learning and memory in mice that have altered LTP.

What sort of rule could better predict whether a certain form of LTP enhancement will lead to improved or reduced spatial memory? One possibility is to take into account the relationship between synaptic strength changes and stimulation frequency as expressed by Bienenstock *et al.* (1982). Perhaps increases of LTP are beneficial only if they occur at intermediate (10–50 Hz) stimulation frequencies while preserving the shift point between LTD and LTP. In any case, it seems clear that merely elevating NMDA receptor-mediated LTP magnitude may not lead to improved neural organization and memory and may in fact be seriously detrimental. The frequency dependence of synaptic strength modifications as well as any changes in intracellular signalling pathways that contribute to LTP are likely to have important clinical implications on development of strategies to improve cognitive function.

References

Abel T, Nguyen PV, Barad M, Deuel TA, Kandel ER & Bourtchouladze R (1997). Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell* **88**, 615–626.

Batschelet E (1981). *Circular Statistics in Biology*. Academic Press, New York.

Bienenstock EL, Cooper LN & Munro PW (1982). Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J Neurosci* **2**, 32–48.

Çavus I & Teyler T (1996). Two forms of long-term potentiation in area CA1 activate different signal transduction cascases. *J Neurophysiol* **76**, 3038–3047.

Çavus I & Teyler TJ (1998). NMDA receptor-independent LTP in basal versus apical dendrites of CA1 pyramidal cell in rat hippocampal slice. *Hippocampus* **8**, 373–379.

Cho YH, Giese KP, Tanila H, Silva AJ & Eichenbaum H (1998). Abnormal hippocampal spatial representations in αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ mice. *Science* **279**, 867–869.

Gerlai R, Henderson JT, Roder JC & Jia Z (1998). Multiple behavioural anomalies in GluR2 mutant mice exhibiting enhanced LTP. *Behav Brain Res* **95**, 37–45.

Grover LM & Teyler TJ (1990). Two components of long-term potentiation induced by different patterns of afferent activation. *Nature* **347**, 477–479.

Jia Z, Agopyan N, Miu P, Xiong Z, Henderson J, Gerlai R, Taverna FA, Velumian A, MacDonald J, Carlen P, Abramow-Newerly W & Roder J (1996). Enhanced LTP in mice deficient in the AMPA receptor GluR2. *Neuron* **17**, 945–956.

Kentros C, Hargreaves E, Hawkins RD, Kandel ER, Shapiro M & Muller RU (1998). Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science* **280**, 2121–2126.

Krucker T, Siggins GR, McNamara RK, Lindsley KA, Dao A, Allison DW, De Lecea L, Lovenberg TW, Sutcliffe JG & Gerendasy DD (2002). Targeted disruption of RC3 reveals a calmodulin-based mechanism for regulating metaplasticity in the hippocampus. *J Neurosci* **22**, 5525–5535.

Kubie JL, Muller RU & Bostock E (1990). Spatial firing properties of hippocampal theta cells. *J Neurosci* **10**, 1110–1123.

Lynch MA (2004). Long-term potentiation and memory. *Physiol Rev* **84**, 87–136.

McHugh TJ, Blum KI, Tsien JZ, Tonegawa S & Wilson MA (1996). Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice. *Cell* **87**, 1339–1349.

Madamba SG, Schweitzer P & Siggings GR (1999). Nociceptin augments K^+ currents in hippocampal CA1 neurons by both ORL-1 and opiate receptor mechanisms. *J Neurophysiol* **82**, 1768–1775.

Mamiya T, Yamada K, Miyamoto Y, Konig N, Watanabe Y, Noda Y & Nabeshima T (2003). Neuronal mechanism of nociceptin-induced modulation of learning and memory: Involvement of *N*-methyl-D-aspartate receptors. *Mol Psychiatry* **8**, 752–765.

Manabe T, Noda Y, Mamiya T, Katagiri H, Houtani T, Nishi M, Noda T, Takahashi T, Sugimoto T, Nabeshima T & Tadeshima H (1998). Facilitation of long-term potentiation and memory in mice lacking nociceptin receptors. *Nature* **394**, 577–581.

Meng Y, Zhang Y, Tregoubov V, Janus C, Cruz L, Jackson M, Lu WY, MacDonald JF, Wang JY, Falls DL & Jia Z (2002). Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Neuron* **35**, 121–133.

Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ & Grant SG (1998). Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**, 433–439.

Nakazawa K, Sun LD, Quirk MC, Rondi-Reig L, Wilson MA & Tonegawa S (2003). Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience. *Neuron* **38**, 305–315.

New DC & Wong YH (2002). The ORL1 receptor: molecular pharmacology and signalling mechanisms. *Neurosignals* **11**, 197–212.

Nishi M, Houtani T, Noda Y, Mamiya T, Sato K, Doi T, Kuno J, Takeshima H, Nukada T, Nabeshima T, Yamashita T, Noda T & Sugimoto T (1997). Unrestrained nociceptive response and disregulation of hearing ability in mice lacking the nociceptin/orphanin FQ receptor. *EMBO J* **16**, 1858–1864.

O'Keefe J & Nadel L (1978). *The Hippocampus as a Cognitive Map*. Clavendon Press, Oxford.

Pineda VV, Athos JI, Wang H, Celver J, Ippolito D, Boulay G, Birnbaumer L & Storm DR (2004). Removal of $G_{i\alpha 1}$ Constraints on adenylyl cyclase in the hippocampus enhances LTP and impairs memory formation. *Neuron* **41**, 153–163.

Ranck JB Jr (1973). Studies on single neurons in drosal hippocampal fromatin and septum in unrestrained rats. *Exp Neurol* **41**, 461–531.

Rotenberg A, Abel T, Hawkins RD, Kandel ER & Muller RU (2000). Parallel instabilities of long-term potentiation, place cells, and learning caused by decreased protein kinase A activity. *J Neurosci* **20**, 8096–8102.

Rotenberg A, Mayford M, Hawkins RD, Kandel ER & Muller RU (1996). Mice expressing activated CaMKII lack low frequency LTP and do not form stable place cells in the CA1 region of the hippocampus. *Cell* **87**, 1351–1361.

Sandin J, Ogren SO & Terenius L (2004). Nociceptin/orphanin FQ modulates spatial learning via ORL-1 receptors in the dorsal hippocampus of the rat. *Brain Res* **997**, 222–233.

Silva AJ (2003). Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. *J Neurobiol* **54**, 224–237.

Somjen GG (2002). Ion regulation in the brain: implications for pathophysiology. *Neuroscientist* **8**, 254–267.

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G & Tsien JZ (1999). Genetic enhancement of learning and memory in mice. *Nature* **401**, 63–69.

Yan J, Zhang Y, Jia Z, Taverna FA, McDonald RJ, Muller RU & Roder JC (2002). Place cell impairment in glutamate receptor 2 mutant mice. *J Neurosci* **22**, RC204–209.

Acknowledgements

F.T. was supported by a Hospital for Sick Children Research Fellowship and a Mt Sinai Hospital Research Fellowship. J.G. was supported by a Canadian Neurotrauma Research Fellowship. The work was supported by a NIH grant NS20686 and an MRC (UK) grant to R.U.M.