Major impairments of glutamatergic transmission and long-term synaptic plasticity in the hippocampus of mice lacking the melanin-concentrating hormone receptor-1.

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**Running head:** Synaptic plasticity in MCH-R1\textsuperscript{-/-} mice

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Abstract

The hypothalamic neuropeptide melanin-concentrating hormone (MCH) plays important roles in energy homeostasis, anxiety and sleep regulation. Since the MCH receptor-1 (MCH-R1), the only functional receptor that mediates MCH functions in rodents, facilitates behavioral performance in hippocampus-dependent learning tasks, we investigated whether glutamatergic transmission in CA1 pyramidal cells could be modulated in mice lacking the MCH-R1 gene (MCH-R1-/-). We found that both AMPA and NMDA receptor-mediated transmission were diminished in the mutant mice compared to their controls. This deficit was, at least in part, explained by a post-synaptic down regulation of these receptors since the amplitude of miniature EPSCs and the NMDA/AMPA ratio were decreased. Long-term synaptic potentiation (LTP) was also impaired in MCH-R1-/- mice. This was due to an altered induction rather than an impaired expression, as repeating induction stimulus restored LTP to a normal magnitude. In addition, long-term synaptic depression (LTD) was strongly diminished in MCH-R1-/- mice. These results suggest that MCH exerts a facilitatory effect on CA1 glutamatergic synaptic transmission and long-term synaptic plasticity. Recently, it has been shown that MCH neurons fire exclusively during sleep and mainly during rapid eye movement sleep. Thus, these findings provide a mechanism by which sleep might facilitate memory consolidation.

Keywords: sleep, LTP, LTD, neuropeptide, glutamate receptor mediated synaptic transmission.
Introduction

The melanin-concentrating hormone (MCH) is a 19 amino acid neuropeptide that has been implicated in several functions such as energy homeostasis and food intake (Nahon 2006; Pissios et al. 2006), anxiety and depression (Roy et al. 2007), and social behaviors (Borowsky et al. 2002). In addition, several studies from our and others laboratories have suggested that MCH may play an important role in the regulation of the sleep states (Verret et al. 2003; Modirrousta et al. 2005; Adamantidis et al. 2008; Ahnaou et al. 2008; Willie et al. 2008). We have shown also that MCH neurons are highly activated during rapid eye movement (REM or paradoxical) sleep (Verret et al. 2003). Importantly, in vivo electrophysiological recordings revealed that MCH neurons fire mostly during REM sleep and occasionally during slow wave sleep (SWS, Hassani et al. 2009). Given that MCH has been also shown to facilitate hippocampal-dependent forms of memory maintenance (Adamantidis and de Lecea 2009), we investigated the possibility that this neuropeptide plays a key role in the synaptic mechanisms by which sleep may facilitate memory consolidation (Benington and Frank 2003; Walker and Stickgold 2006; Rasch and Born 2007).

The MCH-producing neurons are located in the zona incerta and the lateral hypothalamus and send extensive projections to many brain areas including hypothalamus itself, neocortex, hippocampus and amygdala (Bittencourt et al. 1992). The distribution of the MCH receptor-1 (MCH-R1, previously known as the orphan G-coupled receptor SLC-1) expression perfectly matches with the MCH-containing projections (Hervieu et al. 2000; Saito et al. 2001). In particular, dense MCH-containing projections and strong MCH-R1 mRNA expression have been detected in the CA1 and CA3 fields of the hippocampus, which have been identified as neural substrates of learning and memory processes (Frankland and Bontempi 2005). Collectively, these data suggest that MCH-R1 may modulate hippocampal
synaptic transmission and plasticity. Here we investigated the effects of MCH on glutamatergic synaptic transmission, long-term potentiation (LTP) and long-term depression (LTD) in the CA1 area using electrophysiological recordings in hippocampal slices from mice lacking the MCH-R1 gene (MCH-R1\(-\)) and their wild-type (WT) controls. A preliminary report of these results has been presented elsewhere (Pachoud et al. 2006).

**Material and methods**

All the procedures were approved by University Lyon1 animal care and use committee (protocol BH 2006-11). MCH-R1\(-\) mice and their WT littermates were generated as previously described (Adamantidis et al. 2005). We used 4-8-week-old animals with a heterogeneous C57BL6/129SV genetic background. Mice were deeply anaesthetised with sodium pentobarbital (150mg/kg, i.p.) and their brains were dissected. Coronal brain slices (300-400\(\mu\)m) were cut with a vibratome (VT1000S, Leica) in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 10 glucose, 1.25 NaH\(_2\)PO\(_4\), 2.5 KCl, 26 NaHCO\(_3\), 1.3 MgCl\(_2\), and 2.5 CaCl\(_2\). Slices were maintained at room temperature for at least 1h in a submerged chamber containing ACSF equilibrated with 95% O\(_2\) and 5% CO\(_2\), and then transferred to a recording chamber continuously superfused with oxygenated ACSF (~2mL/min). A knife section was made on each slice before recording to separate the CA1 and CA3 regions. All recordings were performed at room temperature (20-25°C) with the gamma-aminobutyric acid (GABA)-A receptor antagonist picrotoxin (100\(\mu\)M, Sigma) added to the ACSF.

Electrical stimulation of Schaffer collaterals (SC) was performed with a bipolar tungsten electrode. Pulses, ranging from 10 to 200\(\mu\)A for a maximal duration of 150\(\mu\)s, were delivered through a stimulus isolator unit (WPI). Stimulating electrode was placed in the CA1 stratum
radiatum, about 100-200μm from the recording site. Field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1 stratum radiatum using borosilicate glass microelectrodes (~1-3MΩ) filled with ACSF and a differential amplifier (WPI).

Baseline fEPSPs were collected at 0.1Hz. We measured the initial slope of fEPSPs to quantify synaptic responses. For each experiment, fEPSP slopes were normalized using average fEPSP slope recorded during the 10-min baseline. Thus, plasticity amplitude was expressed as a percentage of the initial level of the synaptic response. LTP was induced by giving high frequency stimulation (HFS) consisted of 100 pulses at 100Hz. For repeated induction experiments, HFS was repeated four times at 5-minute intervals.

I/O curves. To construct input/output (I/O) curves, we measured the average fEPSPs in response to increasing stimulus intensities. Each average fEPSP consisted of five successive responses to the same intensity. We then plotted each average fEPSP (in mV/ms) against the stimulus intensity that elicited it (in μA), and proceeded to linear fitting of the scatter plot. The slope of the linear curve (I/O curve slope) was taken as an index of synaptic efficacy.

Whole-cell voltage-clamp recordings of CA1 pyramidal cells (low-pass filtered at 2kHz) were carried out using the Axopatch200B amplifier (Axon Instruments) under visual control with an infrared differential interference contrast microscope (BX50WI, Olympus). The patch pipettes were made of borosilicate glass (~5-7MΩ) filled with the following internal solution (in mM): 120 Cs-gluconate, 2 MgCl₂, 10 phosphocreatine, 5 EGTA, 0.5 CaCl₂, 10 HEPES, 4 NaGTP and 0.4 Mg GTP (300mOsm, pH 7.2). Neurobiotin (0.5%, Vector) was routinely added to the patch pipette solution to confirm the morphological identification of pyramidal cells. The neurobiotin labeling was proceeded with avidin conjugated to Texas red D. Labeled neurons were examined by using a fluorescent microscope (Zeiss). Series resistance of the whole-cell patch clamp recordings was monitored continuously for stability (<20% change).
The series resistance mean was not different between mutant and WT mice. A measured liquid junction potential of 12mV was taken into account when setting the holding potentials.

**Evoked EPSCs in strontium.** α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR)-mediated currents were isolated in voltage clamp (holding potential: Vh=-80mV) using the N-methyl-D-aspartate receptor (NMDAR) antagonist D-(-)-2-Amino-5-phosphonopentanoic acid (D-APV, 50µM, Tocris). In each slice, evoked excitatory postsynaptic currents (EPSCs) were first elicited in the ACSF. Calcium (CaCl₂) was then replaced by strontium (SrCl₂, 3mM), which caused miniature, asynchronous EPSCs (aEPSCs) to appear. Measurements were made after 20min of ACSF-SrCl₂ perfusion when asynchronous transmission had stabilized. aEPSCs occurring 10–500ms after onset of the evoked EPSC were analyzed. This analysis window was chosen to minimize contamination by spontaneous EPSCs. Around 200-250 events of aEPSCs were analyzed in each cell using the “Mini Analysis Program” (Synaptosoft).

**Paired-pulse facilitation (PPF) ratio.** The PPF ratio was computed as follow: 
\[(P2/P1)*100\] where P2 was the amplitude of the response (slope for fEPSP, peak amplitude for EPSC) for the second stimulus and P1 was the amplitude of the response of the first stimulus.

**NMDA/AMPA ratio.** For the assessment of the NMDA/AMPA ratio, evoked EPSCs were first recorded at a holding potential of Vh=-80mV to assess AMPAR-mediated responses. Then NMDAR-mediated responses were recorded at Vh=+40mV in presence of the selective AMPAR antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione (NBQX, 5-10µM, Tocris).

Data acquisition and analysis were done blind to genotype. On- and off-line data analyses were carried out using Acquis1 (G. Sadoc, CNRS-ANVAR, France). All summary data were expressed as mean+/−S.E.M., with n representing the number of mice used. When
comparing different groups, statistical significance was assessed using the Mann-Whitney U-
test (MW test) and ANOVA test. When assessing plasticity inside a group, paired t test was
used. All statistical tests were performed with Statistica software (StatSoft, Inc.) and
significance was set at p<0.05.

Results

Basal synaptic transmission at Schaffer collaterals to CA1 pyramidal cell synapses in
MCH-R1⁻/⁻ mice

We performed a series of experiments to search for putative synaptic impairments in MCH-
R1⁻/⁻ mice. First, we carried out the I/O curve of Schaffer collaterals to CA1 (SC-CA1)
synapses to assess basal synaptic transmission using field excitatory postsynaptic potential
(fEPSP) recordings in both strains (Fig. 1A). We observed a two fold decrease in the I/O
curve slopes of MCH-R1⁻/⁻ compared to WT animals (p=0.04, MW test, n=9; Fig. 1A). We
further assessed basal synaptic transmission in MCH-R1⁻/⁻ mice by recording AMPA receptor
(AMPAR)-mediated excitatory postsynaptic currents (EPSCs) in CA1 pyramidal cells (Vh=-
80mV) using the whole-cell voltage-clamp method (Fig. 1D-F). We replaced extracellular
calcium with strontium, which suppresses synchronous vesicle release and enhances
prolonged, asynchronous release from stimulated axon terminals. In strontium, evoked EPSCs
consist of discrete, miniature EPSC-like events (aEPSCs) that represent responses to single,
asynchronously released vesicles from stimulated afferents (Goda and Stevens 1994). Thus,
by stimulating Schaffer collaterals in strontium and D-APV containing ACSF, we were able
to measure quantal size and content at SC-CA1 pyramidal cell synapses in relative isolation
from other inputs (see Methods; Fig. 1D). These experiments revealed that the amplitude of
aEPSCs was significantly reduced in MCH-R1<sup>−/−</sup> mice compared to WT mice (16.7±1 vs 23.1±2.7pA, p=0.02, MW test, n=8 & 10 respectively; Fig. 1E, F). The frequency of aEPSC was also reduced in the mutant mice compared to WT mice (8.2±1.7 vs. 23.7±4.6Hz, p=0.04, MW test; Fig. 1E, F). One possible explanation of these results could be differences in the neuronal types recorded in both strains. However, this possibility seems ruled out since the membrane resistance (calculated by a pulse of -5mV at Vh=-80mV) was not significantly different between MCH-R1<sup>−/−</sup> and WT mice (328±31 vs. 309±43MΩ, p=0.5, MW test, n=11) and all recorded neurons identified by intracellular labeling with neurobiotin showed typical pyramidal cell morphology. Thus, altogether these results indicate an alteration of AMPAR-mediated transmission at SC-CA1 synapses in MCH-R1<sup>−/−</sup> mice.

We then specifically assessed presynaptic function in the mutant mice by testing whether paired-pulse facilitation (PPF) was modulated at SC-CA1 synapses. PPF is a form of short-term synaptic plasticity providing an indirect measure of glutamate release probability at these synapses (Dobrunz and Stevens 1997). The PPF ratio of fEPSPs recorded in control ACSF (2.5mM of CaCl<sub>2</sub>) was computed at different inter-stimulus intervals (ISI: 10, 50, 100, 300 and 1000ms). The PPF ratio was not significantly different between MCH-R1<sup>−/−</sup> and WT mice for all the different ISIs tested (at 50-ms ISI, PPF ratio was 179±7% in MCH-R1<sup>−/−</sup> and 169±9% in WT; p=0.54, MW test, n=7; Fig. 1B). In addition, we quantified the PPF ratio of AMPAR-mediated response in voltage-clamp mode for a 50-ms ISI. Again, we found that PPF was not different in MCH-R1<sup>−/−</sup> and WT mice (at 50-ms ISI, 182±14% vs. 220±25%, p= 0.13, MW test, n=7; Fig. 1C).

**NMDA receptor-mediated synaptic transmission in MCH-R1<sup>−/−</sup> mice**
We then assessed NMDA receptor (NMDAR)-mediated synaptic currents in CA1 pyramidal cells from WT and MCH-R1^{−/−} mice by quantifying the NMDA/AMPA ratio (Fig. 2A, B). The NMDAR/AMPAR ratio was significantly reduced in MCH-R1^{−/−} mice compared to WT mice (94+/−16 vs. 206+/−50%; p=0.03, MW test, n=16 & 13 respectively). In the hippocampus, NMDAR-mediated synaptic responses depend on both NR2A and NR2B subunits. The NR2B-type NMDAR can be assessed pharmacologically by determining the sensitivity of total NMDAR EPSC to the NR2B selective antagonist ifenprodil (3-6µM; Fig. 2C, D). By comparing the remaining NMDAR EPSC to the total NMDAR EPSC we computed the NR2B/NR2A ratio. We found a non-significant decrease in the NR2B/NR2A ratio of the MCH-R1^{−/−} mice compared to WT mice (30.4+/−6 vs. 41.7+/−5%; p=0.18, MW test, n=16 & 18 respectively). Thus, altogether our results suggest a decrease in both AMPAR and NMDAR mediated synaptic transmission in MCH-R1^{−/−} mice.

**LTP characterization in MCH-R1^{−/−} mice**

We then examined whether the disruption of the MCH-R1 gene had an effect on synaptic plasticity by comparing the LTP in CA1 from brain slices of MCH-R1^{−/−} and WT mice (Fig. 3). Given the altered I/O relationship in the mutant mice, we took care of adjusting the stimulus intensity to elicit similar fEPSPs during the baseline prior to all plasticity protocols (Table 1). Average baseline fEPSPs in slices of MCH-R1^{−/−} and WT mice were not significantly different in LTP experiments using a single high frequency stimulus (HFS, p=0.619, MW test). The steady state current activated during the HFS was also not different in MCH-R1^{−/−} and WT mice (0.49+/−0.1 vs. 0.37+/−0.07mV, p=0.41, MW test). In these conditions, cooperativity should be similar in all experiments. A single train of HFS resulted in a sustained increase of the fEPSP slope in WT mice (167+/−13%, p=0.002, paired t test, n=12; Fig. 3A, C). In MCH-R1^{−/−} mice, HFS caused only a weak non-significant increase in
the fEPSP slope (114+/−8%, p= 0.158, paired t test, n= 14; Fig. 3B, C). The average LTP amplitude measured 55-65min after HFS was 53% smaller in MCH-R1−/− compared to WT mice (p=0.0001, MW test; Fig. 3C, D). Male and female mutant mice exhibited the same LTP impairment (ANOVA: genotype p=0.006, gender p=0.42, genotype*gender p=0.69; Table 2), suggesting that plasticity amplitude was influenced by genotype but not gender.

We then tested whether LTP in MCH-R1−/− mice could be rescued by pharmacological enhancement of NMDAR activity. D-serine and glycine are co-agonists at the strychnine-insensitive glycine site of the NMDAR (Johnson and Ascher, 1987) and it has been shown that both are powerful modulators of LTP. Bath application of either D-serine (10-30µM; Fig. 3C, D) or glycine (200µM; data not shown) failed to restore LTP amplitude in slices from mutant mice (LTP amplitude compared in slices from MCH-R1−/− mice with and without D-serine: p=0.72, MW test, n=4).

We further studied whether the inactivation of MCH-R1 affects the induction or the expression process of LTP by using a protocol with repeated HFS known to induce the late form of LTP (L-LTP; Fig. 4). We adjusted the stimulus intensity to elicit similar fEPSPs in MCH-R1−/− and WT mice during the 10-min baseline preceding four HFS (p=0.093, MW test; Table 1). Interestingly, in both mouse strains the amplitude of LTP induced by four HFS was highly significant when measured 55-65min (WT: 161+/−18%, p=0.006, paired t test, n=10; MCH-R1−/−: 166+/−18%, p=0.002, paired t test, n=12; Fig. 4A-C) and 115-125min (WT: 171+/−25%, p= 0.007, MW test; MCH-R1−/−: 170+/−22%, p= 0.002, MW test; Fig. 4A-D) after the tetani. Moreover, there was no significant difference between LTP magnitude in WT and MCH-R1−/− mice at the early (p=0.87, MW test, at 55-65min) and late (p=0.9, MW test, at 115-125min) time course of LTP. Male and female mutant mice displayed similar plasticity amplitudes after the four HFS (ANOVA: genotype p=0.80, gender p=0.52, genotype*gender p=0.62; Table 2). Finally, we also verified that another mechanism did not underlie the LTP...
induced with repeated HFS in the MCH-R1/-/- mice. In the presence of the NMDAR blocker D-APV (100-150µM), repeated HFS was unable to induce a significant LTP in the MCH-R1/-/- mice (117+/−14 %, n=3; data not shown). Therefore, the restoration of LTP by repeated HFS suggests that the MCH-R1-gene deletion selectively altered induction but not expression of the NMDAR-dependent form of LTP at SC-CA1 pyramidal cell synapses.

**LTD characterisation in MCH-R1/-/- mice**

NMDARs also play a critical role in the induction of LTD by low frequency stimulation (LFS) in CA1 (Malenka and Bear, 2004). Given the reduction of NMDA currents in MCH-R1/-/- mice, we wondered whether NMDAR-dependent LTD was also impaired in these mice (Fig. 5). LFS caused a long-lasting depression of fEPSP slope in WT slices (77+/−5%, p=0.021, paired t test; Fig. 5A, C). In MCH-R1/-/- slices, no significant LTD was observed (97+/−6%, p=0.087, paired t test; Fig. 5B, C). The fEPSP slope was transiently depressed but returned to the baseline level within 20min. Finally, 65-75min after LFS, LTD amplitude was significantly reduced in MCH-R1/-/- compared to WT animals (p=0.02, MW test; Fig. 5C, D). Male and female mutant mice exhibited similar LTD impairment (ANOVA: genotype p=0.02, gender p=0.45, genotype*gender p=0.51; Table 2). Altogether, these results indicated that the two major forms of hippocampal long-term synaptic plasticity, LTP and LTD, were impaired in the MCH-R1/-/- mice.

**Discussion**

The biological effects of MCH in mice are exclusively mediated by the MCH-R1 (Tan et al. 2002). Compared to MCH precursor knockout mice, the genetic model we used does not lack other peptides of unknown function encoded at the MCH gene locus, including the
neuropeptides NEI and NGE, the MCH-gene-overprinted-polypeptide (MGOP) and antisense-
RNA-overlapping-MCH gene (AROM) (Allaeys et al. 2004; Griffond and Baker 2002). Thus, the MCH-R1−/− mouse model allowed us to selectively assess the role of the MCH peptide in SC-CA1 glutamatergic transmission. In the present study, we found that the lack of functional MCH-R1 caused a major impairment of glutamate synaptic transmission, LTP induction and LTD in the CA1 region of the hippocampus. Thus, in addition to the suggested inhibitory properties of the MCH neuropeptide (Gao and van den Pol 2001; Rao et al. 2008), activation of the MCH-R1 may also facilitate glutamatergic transmission and long-term synaptic plasticity. These actions could underlie the effects of the MCH system on memory consolidation (Adamantidis et al. 2005; Monzon et al. 1999). These results led to the hypothesis that MCH as well as other hormones involved in food intake and metabolism may enable animals to adopt optimal food-seeking strategies by allowing them to remember food locations and to recall those locations (Moran and Gao 2006).

Before interpreting the results, we should examine whether endocrine alterations in MCH-R1−/− mice may affect synaptic physiology. The plasma level of leptin is lower in MCH-R1−/− than in WT mice (Chen et al. 2002; Marsh et al. 2002). Leptin has been shown to facilitate hippocampal synaptic plasticity through an enhancement of the synaptic NMDAR-mediated current (Durakoglugil et al. 2005; Shanley et al. 2001). In these conditions it seems that the reduction of NMDAR currents and the alteration of LTP that we found in MCH-R1−/− mice could be a consequence of the leptin deficiency that affects these animals. However, we observed the same impairments of LTP in MCH-R1−/− mice from both genders (Table 2) whereas male but not female mutant mice exhibit hypoleptinemia. Thus the synaptic defects of MCH-R1−/− mice cannot be explained by a deficit in leptin. Plasma corticosterone concentration is also slightly elevated in MCH-R1−/− mice (Marsh et al. 2002). Although it is known that corticosterone interacts with synaptic plasticity in CA1 (Yang et al. 2004; Maggio...
and Segal 2007), it is unlikely that the deficits we observed in MCH-R1<sup>−/−</sup> mice are due to an indirect effect of stress because stress or application of corticosterone have no effect on AMPAR-mediated transmission and LTD in CA1 (Maggio and Segal 2007). Thus, altogether our results suggest that the deficits in synaptic transmission and plasticity observed in MCH-R1<sup>−/−</sup> mice are not a consequence of indirect effects of the altered levels of leptin and/or corticosterone.

AMPAR and NMDAR are two major classes of glutamatergic receptors in the CA1 region of the hippocampus. Our results suggest a large decrease in the synaptic NMDAR current in MCH-R1<sup>−/−</sup> mice compared to WT mice. It has been shown that exogenous NMDA application (in the presence of the sodium channel blocker tetrodotoxin), which activates mostly extrasynaptic NMDAR, induced a lower depolarization and less calcium spikes in CA1 pyramidal cells of MCH-R1<sup>−/−</sup> mice compared to their controls (Adamantidis et al. 2005). Altogether, these results suggest that both synaptic and extrasynaptic NMDAR function is altered in MCH-R1<sup>−/−</sup> mice. In addition, NR1 mRNA expression was found to be decreased in CA1 of MCH-R1<sup>−/−</sup> mice (Adamantidis et al. 2005). Thus MCH-R1 activation may be involved in the control of NMDAR synthesis. Surprisingly, we found a decrease in strontium-induced miniature AMPAR-mediated EPSC (aEPSC) amplitude suggesting a reduction in quantal size (Goda and Stevens 1994). Frequency of aEPSCs was also significantly decreased suggesting a presynaptic expression mechanism (Bolshakov and Siegelbaum 1994). What could be the simplest mechanism which may explain these findings? A plausible hypothesis is that MCH-R1<sup>−/−</sup> mice may express LTD in CA1 at basal level. Interestingly, the NMDAR-dependent form of CA1 LTD (NMDAR-LTD) is also associated with a decrease in both amplitude and frequency of aEPSCs (Oliet et al. 1996), and with a decrease in NMDAR-mediated synaptic transmission (Malenka and Bear 2004). In addition, no modulation of the paired-pulse facilitation ratio has been reported during NMDAR-LTD (Xiao et al. 1995;
Santschi and Stanton 2003). Several functional alterations we found at MCH-R1⁻/⁻ naïve synapses are similar to the synaptic changes associated with NMDAR-LTD. If MCH-R1⁻/⁻ naïve synapses express a basal LTD, thus the defects of SC-CA1 synaptic transmission in the mutant mice are likely to be related to a down regulation of clusters of AMPAR and NMDAR, a proposed mechanism for NMDAR-LTD expression. The lack of LTD in MCH-R1⁻/⁻ mice may provide further support to this hypothesis. Since both NMDAR and AMPAR-mediated synaptic responses were decreased, it is also possible that MCH-R1⁻/⁻ mice have undergone a synaptic reorganization at a morphological level. To our knowledge, the morphology and the density of synapses have not yet been addressed in MCH-R1⁻/⁻ mice. However, some alterations could be expected since a recent study showed that MCH treatment promotes neurite outgrowth in cell culture of neuroblastoma presumably by activating the MAP kinase cascade (Cotta-Grand et al. 2009). In transfected cells, MCH-R1 couples to different G proteins, leading to multiple effects including an increase in intracellular free Ca²⁺ and the activation of MAP kinase cascade (Pissios et al. 2003). The activation of MAP kinase cascade may positively regulate dendritic protein synthesis by controlling translation (Kelleher et al. 2004). Thus, the alterations of glutamate-mediated synaptic transmission that we found in the mutant mice could be due to a prolonged lack of activation of MAP kinase cascade.

LTP was impaired in MCH-R1⁻/⁻ compared to WT mice, but repeated HFS rescued LTP to a normal magnitude suggesting that LTP expression was not affected. Thus, our findings indicate that deletion of the MCH-R1 gene causes an impairment of LTP induction. The cause of LTP induction impairment was unlikely to be a decrease in cooperativity (Andersen 1987). Cooperativity relies on the activation of a sufficient number of afferent fibers that cooperate to induce LTP during the high frequency train. We adjusted the baseline fEPSPs for all plasticity experiments in order to obtain the same amount of cooperativity (these experiments
were done blind to the genotype). A previous study reported that in vivo MCH application lowers the induction threshold of LTP in the rat dentate gyrus (Varas et al. 2003).

Interestingly, this effect was delayed as it was observed 48h after in vivo MCH injection.

Thus, the higher induction threshold of LTP we found in MCH-R1\(^{-/-}\) mice may be caused by a chronic lack of MCH-R1 activation leading to molecular alterations of the plasticity induction mechanisms. In CA1, the NMDAR-mediated synaptic transmission is required for LTP and L-LTP as demonstrated by several studies (Malenka and Bear 2004). Given the key role of NMDAR activation in CA1 LTP induction, we propose that the decrease in NMDAR mediated synaptic transmission is likely to explain, at least in part, the alteration in LTP induction that we observed. However, it has also been shown that the GluR1 subunit of AMPAR is required for CA1 LTP (Zamanillo et al. 1999; Malenka and Bear 2004). We cannot discard the possibility that a change in AMPAR subunit composition in the mutant mice could occur and add to the LTP impairment we observed.

Interestingly, we showed that LTD is also impaired in MCH-R1\(^{-/-}\) mice. Thus, our results do not support a role for MCH-R1 in the regulation of hippocampal metaplasticity (Abraham and Bear 1996) as the mutant mice did not present a shift of the induction threshold of LTP toward LTD. As LTP, CA1 LTD induced by LFS depends on NMDAR activation. The alteration of LTD in the mutant mice could be a consequence of the reduced NMDA function, or it could be related to impairment in the molecular cascades controlling LTD such as protein phosphatases (Malenka and Bear 2004). It has been shown that MCH-R1 signalling might reduce phosphorylation of AMPAR subunit GluR1 at Ser845 in the nucleus accumbens via the activity of calcineurin (Georgescu et al. 2005; Sears et al. 2006). Given that dephosphorylation of Ser845 plays a key role in CA1 LTD (Lee et al. 2000), the lack of MCH-R1 activation in the mutant mice could have led to the impairment of LTD. Additional molecular studies are needed to explain this synaptic defect.
Implications for the physiological action of MCH during sleep

Since MCH neurons are mainly activated during REM sleep (Verret et al. 2003; Hassani et al. 2009), the present results suggest an important role for MCH in the regulation of hippocampal synaptic plasticity during this vigilance state. LTP is considered as a potential mechanism for memory consolidation and both REM sleep and SWS play important roles in memory maintenance (Benington and Frank 2003; Frankland and Bontempi 2004; Walker and Stickgold 2006). We and others have shown that REM sleep deprivation impaired LTP and NMDAR mediated transmission (McDermott et al. 2003; Ravassard et al. 2009). Moreover, our recent study suggests that REM sleep facilitates AMPAR-mediated synaptic transmission (Ravassard et al. 2009). One potential mechanism by which LTP induction and synaptic transmission could be facilitated during sleep is the phosphorylation of ERK (Ravassard et al., 2009), a key enzyme of MAP kinases cascade. As discussed above, this cascade might be the main signalling pathways underlying the MCH-R1 activation. Thus, based on the results of the present study, we hypothesize that MCH is one of the key mechanisms underlying the REM sleep-induced facilitation of LTP and synaptic transmission. It will be crucial to determine whether the activation of hippocampal MCH-R1 is required for REM sleep-dependent facilitation of memory consolidation. Another key issue is to characterize the role of MCH on the hippocampal replays of neuronal activity during sleep after learning (Louie and Wilson 2001; Rasch and Born 2007).

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References


Sears RM, Yeckel M, Dileone RJ. Examination of MCH1R signaling on the biochemical and cellular properties of medium spiny neurons in the nucleus accumbens shell. *Society For Neuroscience annual meeting abstract*, Atlanta, USA, 2006.


**Figure Legends**

**Figure 1.** AMPAR-mediated SC-CA1 synaptic transmission was altered in MCH-R1<sup>−/−</sup> mice. **A.** Average I/O curves from WT and MCH-R1<sup>−/−</sup>. The average slope values in MCH-R1<sup>−/−</sup> mice (black diamonds) were significantly decrease compared to WT (empty circle)(for MCH-R1<sup>−/−</sup>, slope=0.023+/−0.003, R=0.998, p<0.0001; for WT: slope=0.046+/−0.006, R=0.998, p<0.001; p=0.04, n=9). Average traces are the superimposed responses to the different stimulus intensities. Scale: 0.6mV / 20ms. **B.** Summary plot of the paired-pulse facilitation (PPF) experiments using LFP recordings. The paired-pulse ratio computed at different inter-stimulus intervals was not significantly different in MCH-R1<sup>−/−</sup> and WT. At 50-ms interval the values of the ratio were 179+/−7% in MCH-R1<sup>−/−</sup> and 169+/−9% in WT (p=0.54, n=7). Scale: 0.2mV / 200ms. **C.** PPF measured on AMPAR-EPSCs was not altered in MCH-R1<sup>−/−</sup> compared to WT mice (PPF ratio for an inter-stimulus interval of 50ms: 182+/−14 vs. 220+/−25%, p=0.13, n=7). Histograms represent the average PPF ratio and the points are the value for individual experiments. Scale: 50pA / 100ms. **D.** Typical aEPSCs recordings
(3 superimposed traces) in a CA1 pyramidal cell of MCH-R1\textsuperscript{-/-} (black diamond) and WT (open circle) mouse showing miniature, asynchronous events evoked under strontium. Scale: 50pA / 250ms. E. Cumulative probability curves of aEPSC peak amplitudes in MCH-R1\textsuperscript{-/-} (thick line) and WT (thin line) cells. aEPSC peak amplitudes were significantly reduced in the mutant mice (187 & 231 events, p<0.0001, Komolgorov-Smirnov test). Superimposed average traces of ~80 miniature EPSCs from a MCH-R1\textsuperscript{-/-} (thick line) and a WT neuron (thin line). Scale: 20pA / 40ms. Lower panel: Cumulative probability curves of aEPSC frequency in the MCH-R1\textsuperscript{-/-} (thick line) and in the WT (thin line) neuron: the frequency of aEPSCs was significantly decreased in the MCH-R1\textsuperscript{-/-} (thick line) compared to the WT (thin line) neuron (p<0.0001, Komolgorov-Smirnov test). F. Average peak amplitude (upper panel) and frequency (lower panel) of the aEPSCs. Both values were significantly lower in MCH-R1\textsuperscript{-/-} mice compared to WT (amplitude: 16.7+/-.1 vs 23.1+/-.27pA, p=0.02. frequency: 8.2+/-.1.7 vs 23.7+/-.4.6Hz, p=0.04). In both panels, the histograms represent the average and the points are the value for individual experiments.

**Figure 2. NMDAR-mediated SC-CA1 synaptic transmission was altered in MCH-R1\textsuperscript{-/-} mice.** A. Average traces (10 sweeps) of evoked EPSCs obtained from two representative CA1 pyramidal cells recorded in a WT (empty circle) and a MCH-R1\textsuperscript{-/-} (black diamond) mouse. Recordings were first done in picrotoxin at holding potential V\textsubscript{h}=-80mV (AMPAR-EPSC) and then at V\textsubscript{h}=+40mV in the presence of 10\textmu M NBQX to isolate the NMDAR-mediated response (NMDAR-EPSC). B. Summary graph of the mean NMDAR/AMPAR ratio obtained by dividing NMDAR-EPSC peak amplitude by AMPAR-EPSC peak amplitude in WT and MCH-R1\textsuperscript{-/-} mice. The values for each individual experiment are also shown. On average, the NMDAR/AMPAR ratio was significantly decreased in CA1 pyramidal cells of MCH-R1\textsuperscript{-/-} compared to WT mice (94+/-.16 vs 206+/-.50%, p=0.03, n= 16 & 13 respectively). C. Average
traces showing typical recordings of NMDAR response in NBQX (Vh=±40mV), in the presence of the NR2B-type NMDAR antagonist ifenprodil (6µM) and in the presence of the NMDAR antagonist D-APV (50µM). D. As shown by the average value of the NR2B/NMDA ratio, a non-significant decrease in NR2B-mediated synaptic transmission was observed in MCH-R1<sup>−/−</sup> compared to WT pyramidal cells (30.4+/−6 vs 41.7+/−5%, p=0.18, n=17 & 18 respectively).

**Figure 3. SC-CA1 LTP was impaired in MCH-R1<sup>−/−</sup> mice.** A, B. Time course of the fEPSP slope in two representative LTP experiments from a WT (A) and a MCH-R1<sup>−/−</sup> (B) slice. LTP was induced by a single HFS (arrow). Sample traces are average of 10 successive sweeps taken 5min before (thin line) and 60min after (thick line) HFS. Scale: 0.1mV / 10ms. C, D. Summary plots of LTP experiments. A 53% decrease in LTP amplitude was observed 55-65min after HFS in MCH-R1<sup>−/−</sup> (n=14) as compared to WT (n=12) mice, suggesting an impairment of LTP in the mutant mice (p=0.0001). Adding D-serine (DS, 10-30µM) to the ACSF did not rescued LTP in MCH-R1<sup>−/−</sup> slices. In these conditions, the average fEPSP after LTP induction was also significantly reduced (p=0.002, n=4) as compared to WT and not significantly different compared to MCH-R1<sup>−/−</sup> slices without D-serine (p=0.72, n=4).

**Figure 4. Repeated HFS restored SC-CA1 LTP in MCH-R1<sup>−/−</sup> mice.** A, B. Time course of the fEPSP slope in two representative repeated HFS experiments from a WT (A) and MCH-R1<sup>−/−</sup> (B) slice. LTP was induced by four HFS repeated at 5-min intervals (arrows). Sample traces are average of 10 successive sweeps taken 5min before (thin line) and 60min after (thick line) HFS. Scale: 0.1mV / 10ms. C, D. The summary plots of repeated HFS experiments show no significant difference in LTP magnitude between WT and MCH-R1<sup>−/−</sup>
mice (MCH-R1\(^{-/-}\): 170+/−22%, WT: 171+/−25%, \(p=0.9\), at 115-125min) suggesting that LTP expression is normal in the mutant mice.

**Figure 5.** SC-CA1 LTD was impaired in MCH-R1\(^{-/-}\) mice. A, B. Time course of the fEPSP slope in two representative LTD experiments from a WT (A) and MCH-R1\(^{-/-}\) (B) slice. LTD was induced by LFS (1Hz, 15min, black bar). Sample traces are average of 10 successive sweeps taken 5min before (thin line) and 60min after (thick line) LFS. Scale: 0.1mV / 10ms. C, D. Summary plots of LTD experiments. A significant reduction of LTD amplitude \(p=0.02\) was observed 65-75min after LFS in MCH-R1\(^{-/-}\) \((n=12)\) as compared to WT \((n=12)\) mice, suggesting an impairment of LTD in the mutant mice.

Table 1. Average fEPSP values during the baseline preceding synaptic plasticity induction. For each experimental condition, we adjusted stimulus intensity to record similar-sized fEPSPs during baseline acquisition. Baseline fEPSP slopes in WT and MCH-R1\(^{-/-}\) mice were not significantly different in the one HFS \((p=0.619)\), four HFS \((p=0.093)\), and LFS \((p=0.795)\) experiments. Thus, the influence of the cooperativity effect on plasticity induction should be similar in WT and MCH-R1\(^{-/-}\) slices.

Table 2. Effects of genotype and gender on the magnitude of synaptic plasticity. Summary of the mean fEPSP slope (normalized relative to baseline value) recorded in male and female mice from both strains following the different induction protocols (1 HFS, 4 HFS and LFS). Male and female mutant mice exhibited a similar pattern of plasticity, as confirmed by ANOVA that revealed a significant effect for genotype but not for sex. Assessment of significant changes of the synaptic response following plasticity induction was made by paired t test inside each group \((p values for this test are shown in the table)\).
Table 1. Average fEPSP values during the baseline preceding synaptic plasticity induction.

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<td>WT</td>
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Table 2. Effects of genotype and gender on the magnitude of synaptic plasticity.

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