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NMDA receptor-dependent metaplasticity at hippocampal mossy fiber synapses

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ABSTRACT (86 words)

Hippocampal mossy fiber synapses (Mf-synapses) have been reported to lack NMDA receptor-dependent LTP of AMPA-EPSCs, unlike conventional glutamatergic synapses. An explanation for this difference may reside in the relatively low amount of NMDA receptors at these synapses. Because Mf-synapses display LTP selective for NMDA receptors, we examined whether this would impact on the plasticity rules at Mf-CA3 synapses in mouse hippocampal slices. Here we show that LTP of NMDA receptors serves as a metaplastic switch making Mf-synapses competent for generating NMDA receptor-dependent LTP of AMPA-EPSCs.

Key words: NMDA receptors, hippocampal mossy fibers, synaptic plasticity, metaplasticity

Ionotropic glutamate receptors of the NMDA type (NMDARs) are widely known to be essential triggers for long-term potentiation (LTP) of excitatory synaptic transmission¹. Not all glutamatergic synapses have been reported to display NMDA receptor-dependent LTP of AMPA-EPSCs. Among these, hippocampal mossy fiber synapses onto CA3 pyramidal cells (Mf-synapses) display several unique features, including pronounced short-term facilitation and a presynaptic form of LTP that is independent of NMDA receptor activation, but no NMDA receptor-dependent LTP of AMPA-EPSCs^{2,3}. Although Mf-synapses are equipped with NMDARs⁴ like the vast majority of glutamatergic synapses, one can hypothesize that the amount of NMDA receptors at Mf-synapses is not sufficient to allow LTP to be triggered. We compared the relative amplitude of NMDA versus AMPA-EPSCs at Mf-synapses and at associational/commissural fiber synapses (A/C synapses), glutamatergic synapses made onto the distal dendrites of CA3 pyramidal cells known to display NMDA receptor-dependent LTP⁵. In support of our hypothesis, we observed a 5-fold difference in NMDA/AMPA ratio (Sup. Fig. 1A, B; $P < 0.01$) (see also⁶).

Recently we and others have described at Mf-synapses a form of LTP expressed by NMDARs but not by AMPARs^{7,8}. We thus examined whether this form of LTP had an impact on the plasticity rules at Mf-synapses by selectively increasing the amplitude of NMDA-EPSCs. We tested two commonly used protocols to trigger NMDA receptor-dependent plasticity, a regular spike-timing-dependent-plasticity protocol (STDP-pairing) and a depolarization-pairing protocol (depo-pairing) (Fig. 1A). In control conditions both protocols induced a clear and long-lasting LTP at A/C synapses (STDP-LTP: $160.2 \pm 13.5\%$ $n=9$; depo LTP: $163.1 \pm 10.5\%$ $n=16$) (Fig. 1B-E, I, J), but not at Mf-synapses (STDP-LTP: $103.7 \pm 9.9\%$ $n=7$; depo LTP: $99.4 \pm 14.5\%$ $n=16$). These results indicate that in control conditions A/C synapses behave like conventional glutamatergic synapses displaying NMDA receptor-dependent LTP whereas Mf-synapses lack NMDA receptor-dependent LTP.

We next tested the hypothesis that prior LTP of NMDARs with the consequent increase in NMDA-EPSCs, may render Mf-synapses competent for expressing conventional NMDA receptor-dependent LTP at Mf-synapses. We first verified that LTP of NMDARs can be induced at Mf-synapses while recording from CA3 pyramidal cells in the current-clamp mode at their resting membrane potential (around -70 mV).

A few bursts of presynaptic stimuli (6 bursts of 6 stimuli at 50 Hz)⁸, reliably induced robust LTP of NMDA-EPSPs (171 ± 17 %, $n=7$), but did not affect durably the peak amplitude of Mf-EPSPs (110 ± 10 %, $n=14$), indicating a lack of potentiation of AMPARs (Sup. Fig. 2A-C). The selective potentiation of NMDARs was particularly evident when monitoring the participation of NMDARs to Mf-EPSPs before and after triggering LTP of NMDARs. The NMDAR antagonist, D-AP5 (50 μ M), which only modestly affected Mf-EPSPs recorded at 1 Hz in control conditions, significantly reduced the decay time course of Mf-EPSPs recorded after LTP of NMDARs (Sup. Fig. 2D-F; Supp. Fig. 3). D-AP5, did not affect Mf-EPSPs recorded at 0.1 Hz, neither in control conditions nor after LTP of NMDARs (Supp. Fig. 2, D-F; Sup. Fig. 3), likely because the depolarization reached at this stimulation frequency is not sufficient to relieve NMDARs from the Mg^{2+} block. Hence, LTP of NMDARs leads to prolonged decay of Mf-EPSPs with no change in their amplitude. Interestingly and as a consequence, there was a significant increase in CA3 pyramidal cell action potential discharge in response to short bursts of Mf stimulation (Sup. Fig. 2G, H, $P < 0.05$) or to sustained 3Hz stimulation (data not shown). This increase in spike transmission depends on NMDARs (Sup. Fig. 2H,I) and is not accompanied with changes in CA3 pyramidal cell intrinsic excitability (Supp. Fig. 4). Thus, LTP of NMDARs amplifies spike transfer and exacerbates the "detonator" properties of Mf-CA3 synapses⁹.

Selective LTP of NMDARs can thus be induced in current-clamp recording conditions at the resting membrane potential. We then tested if prior LTP of NMDARs influenced the possibility to generate NMDA receptor-dependent LTP of Mf-EPSPs using the protocols described above (Fig. 1A). Twenty minutes after triggering LTP of NMDARs, both pairing protocols (STDP-pairing and depo-pairing) induced a sustained LTP at Mf-synapses (STDP-pairing: 190.6 ± 25.6 % $n=13$, $P < 0.05$; depo-pairing: 159.4 ± 16.6 % $n=10$, $P < 0.05$) (Fig. 1F-J). No depo-pairing LTP was observed in the presence of the mGluR5 antagonist, MPEP (10 μ M), which blocks induction of LTP of NMDARs⁸ (Sup. Fig. 5). We have previously shown that high frequency stimulation (HFS) which classically induces presynaptic LTP at Mf-synapses can also induce LTP of NMDARs⁸. Accordingly, we found that the HFS protocol primed Mf-synapses to express depo-pairing LTP, as indicated by the additional potentiation found on top of the potentiation induced by the HFS protocol ($P < 0.05$, Sup. Fig. 6).

The observed STDP-LTP of Mf-EPSPs after prior induction of LTP of NMDARs is dependent on the activation of NMDARs. Indeed, bath application of D-AP5 after

induction of LTP of NMDARs, blocked induction of LTP ($96.5 \pm 10.0 \%$, $n=11$, $P < 0.05$) (Fig. 2A, B, C). To evaluate the dependence of STDP-LTP on postsynaptic calcium increase, we used a patch/re-patch procedure, because buffering calcium by itself prevents LTP of NMDARs. We patched CA3 pyramidal cells, induced LTP of NMDARs and carefully removed the patch pipette. We then re-patched the same CA3 pyramidal cell with either the same internal solution or a solution containing BAPTA (20 mM). The patch/re-patch approach with twice the control internal patch solution did not affect induction of STDP-LTP at Mf-synapses (153.7 ± 20.2 , $n=9$) (Fig. 2D, F and I). However, inclusion of BAPTA in the intracellular solution while re-patching cells abrogated induction of STDP-LTP suggesting the need for postsynaptic calcium rise (81.3 ± 7.8 , $n=9$, $P < 0.01$) (Fig. 2E, F and I).

If the prior potentiation of NMDARs is the critical factor for the induction of NMDA receptor-dependent STDP at Mf-synapses, directly boosting NMDAR currents should reveal this form of plasticity in control conditions. To test this hypothesis, we bath applied the NMDAR co-agonist D-serine (100 μ M), which significantly increased Mf-NMDA-EPSCs to a level similar to LTP of NMDARs (Supp. Fig. 7). In these conditions, the STDP protocol induced LTP of Mf-EPSPs ($157.6 \pm 18.9\%$, $n=12$) (Fig. 2F-H). We obtained the same results in voltage-clamp conditions using the depolarizing protocol (Sup. Fig. 8). The STDP-LTP recorded at Mf-synapses in the presence of D-serine was also dependent on NMDARs since it was blocked by bath application of D-AP5 (50 μ M) ($97 \pm 3\%$, $n=9$, $P < 0.05$) (Fig. 2G-I) or by adding 1 mM MK-801 to the intracellular solution (Sup. Fig. 9, $P < 0.01$). To exclude the possibility that the observed Mf-LTP in the presence of D-serine is linked to some side effect of D-serine on other target than NMDARs we used another approach to boost NMDARs. We have previously observed that adenosine A_{2A} receptors activation selectively increases NMDARs-EPSCs at Mf-synapses⁸. Likewise, in the presence of the A_{2A} receptor agonist, CGS 21680 (30 nM), the STDP protocol induced LTP of Mf-EPSPs (Sup. Fig. 10). These experiments strongly suggest that boosting NMDARs is indeed the factor responsible for the metaplastic switch rendering Mf-synapses competent for NMDA receptor-dependent LTP.

We performed additional experiments to exclude the possibility that the observed potentiation of Mf-EPSPs was in fact related to polysynaptic activation and contamination of Mf-EPSCs by A/C EPSCs. We first recorded Mf-EPSCs in the presence 10-30 nM TTX to minimize polysynaptic activity, and we probed depolarizing

LTP of Mf-EPSCs either after bath-applied D-serine to boost NMDARs, or after LTP of NMDARs. Mf-EPSCs recorded in these conditions showed a small rundown over time (Sup. Fig. 11A-F). Nonetheless, in these conditions, the depo-pairing protocol induced a clear LTP of Mf-EPSCs (Sup. Fig. 11A-F; $P < 0.01$). We also found that depo-pairing LTP of Mf-EPSCs in the presence of D-serine was completely blocked by nifedipine, an L-type calcium channel blocker, whereas nifedipine did not affect LTP at A/C synapses (Sup. Fig 11G-I). Altogether these results indicate that there is likely minimal contamination of Mf-EPSCs by polysynaptic activation, and that the potentiation of Mf-EPSCs/EPSPs cannot be attributed to potentiated A/C synaptic currents.

Hence we have shown that LTP of NMDARs acts as a metaplastic switch allowing Mf-synapses to express NMDA receptor-dependent LTP of AMPA-EPSCs. At the post-synaptic level Mf-CA3 synapses thus behave as conventional glutamatergic synapses, provided that they are primed by presynaptic bursting activity triggering a metaplastic switch. This metaplastic switch represents a powerful mechanism allowing strong reinforcement of Mf-synapses that undergo recurrent activation in an NMDA receptor-dependent manner. Such reinforcement may play an important role during NMDA- dependent learning tasks that involve the dentate gyrus. Further experiments should explore the precise contribution of Mf-NMDARs in the known behavioral effects attributed to NMDARs present in CA3 pyramidal cells ¹⁰⁻¹².

Figure 1 – LTP of NMDARs acts as a metaplastic switch at hippocampal Mf-CA3 synapses.

(A) Illustrative scheme of the different pairing protocols used to trigger NMDA receptor-dependent LTP. (B) In control conditions a STDP protocol consisting of 30 presynaptic stimuli each paired with three postsynaptic action potentials (STDP-pairing) induces LTP of EPSPs at Associational/Commissural (A/C) but not at Mf-synapses (C) Representative traces of Mf-EPSPs and A/C-EPSPs before and after the pairing STDP protocol. (D) Pairing Mf stimulation with a depolarization of CA3 pyramidal cells to 0 mV (depo-pairing) induces LTP of EPSCs at A/C synapses but not at Mf-synapses. (E) Representative traces of Mf-EPSCs and A/C-EPSCs synapses before and the depo-pairing protocol. (F,H) LTP of NMDARs renders Mf-synapses responsive to both STDP and depo-pairing protocols. (G) Illustrative traces of Mf-EPSPs and Mf-EPSCs showing the effectiveness of the two pairing protocols in inducing LTP at Mf-synapses following induction of LTP of NMDARs. (I) Average potentiation of Mf-EPSP with an STDP

protocol at A/C synapses and at Mf-synapses in control conditions and at Mf-synapses primed by LTP of NMDARs **(J)** Average potentiation of Mf-EPSCs with a depo-pairing protocol at A/C synapses and at Mf-synapses in control conditions and at Mf-synapses primed by LTP of NMDARs. Error bars represent s.e.m.

Figure 2 – STDP-LTP at Mf-synapses is dependent on NMDARs and postsynaptic calcium rise. Boosting NMDARs with D-serine renders Mf-synapses responsive to STDP-protocols.

(A) STDP observed after induction of LTP of NMDARs at Mf-synapses is blocked by D-AP5 (50 μ M). **(B)** Representative traces of Mf-EPSPs following induction of LTP of NMDARs illustrating the block of STDP-LTP by D-AP5. **(C)** Average potentiation of Mf-EPSP with the STDP protocol at Mf-synapses primed by LTP of NMDARs in the presence or absence of D-AP5. **(D)** CA3 pyramidal cells were patched and after triggering LTP of NMDARs the patch pipette was carefully removed. The same CA3 pyramidal cells were then re-patched and the STDP pairing protocol was applied. Re-patched cells exhibited STDP-LTP. **(E)** When CA3 pyramidal cells were re-patched with an intracellular solution containing BAPTA (20 mM) no STDP-LTP was observed. **(F)** Representative traces of Mf-EPSPs obtained from re-patched CA3 pyramidal cells illustrating the potentiation induced by the STDP protocol in control conditions and the lack of potentiation when 20 mM BAPTA was included in the patch pipette. **(G)** In the presence of D-serine the STDP-protocol induced LTP at Mf-synapses. **(H, I)** Representative traces of Mf-EPSPs illustrating STDP-LTP induced in the presence of D-serine and its block by D-AP5. **(J)** Average values for experiments illustrated in D-I. Error bars represent s.e.m.

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Authors' contribution

NR, CB, FL and MC performed the experiments, NR and CM designed the experiments and wrote the manuscript.

Supplementary Information Titles

Journal: Nature Neuroscience

Article Title:	NMDA receptor-dependent metaplasticity at hippocampal mossy fiber synapses
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Supplementary Item & Number	Title or Caption
Supplementary Figure 1	NMDA/AMPA ratio is smaller at Mf-synapses than at A/C synapses.
Supplementary Figure 2	LTP of NMDARs affects EPSP time course and facilitates spike transfer at hippocampal Mf-CA3 synapses.
Supplementary Figure 3	Time course and amplitude of Mf-EPSPs before and after LTP of NMDARs
Supplementary Figure 4	Induction of LTP of NMDARs does not change CA3 pyramidal cell intrinsic excitability
Supplementary Figure 5	Metaplasticity is prevented by the mGluR5 antagonist MPEP
Supplementary Figure 6	Metaplasticity can also be induced by high frequency stimulation (HFS) protocols known to trigger presynaptic LTP at Mf-synapses
Supplementary Figure 7	D-serine potentiates NMDARs at Mf-synapses
Supplementary Figure 8	D-serine mimics the effects of LTP of NMDARs at Mf-synapses
Supplementary Figure 9	STDP-LTP at Mf-synapses is blocked by intracellular inclusion of MK-801
Supplementary Figure 10	Potentiation of NMDARs with the adenosine A _{2A} receptor CGS 21680 renders Mf-synapses responsive to the STDP protocol
Supplementary Figure 11	LTP observed at Mf-synapses cannot be explained by contamination of synaptic responses by A/C EPSCs

Figure 1

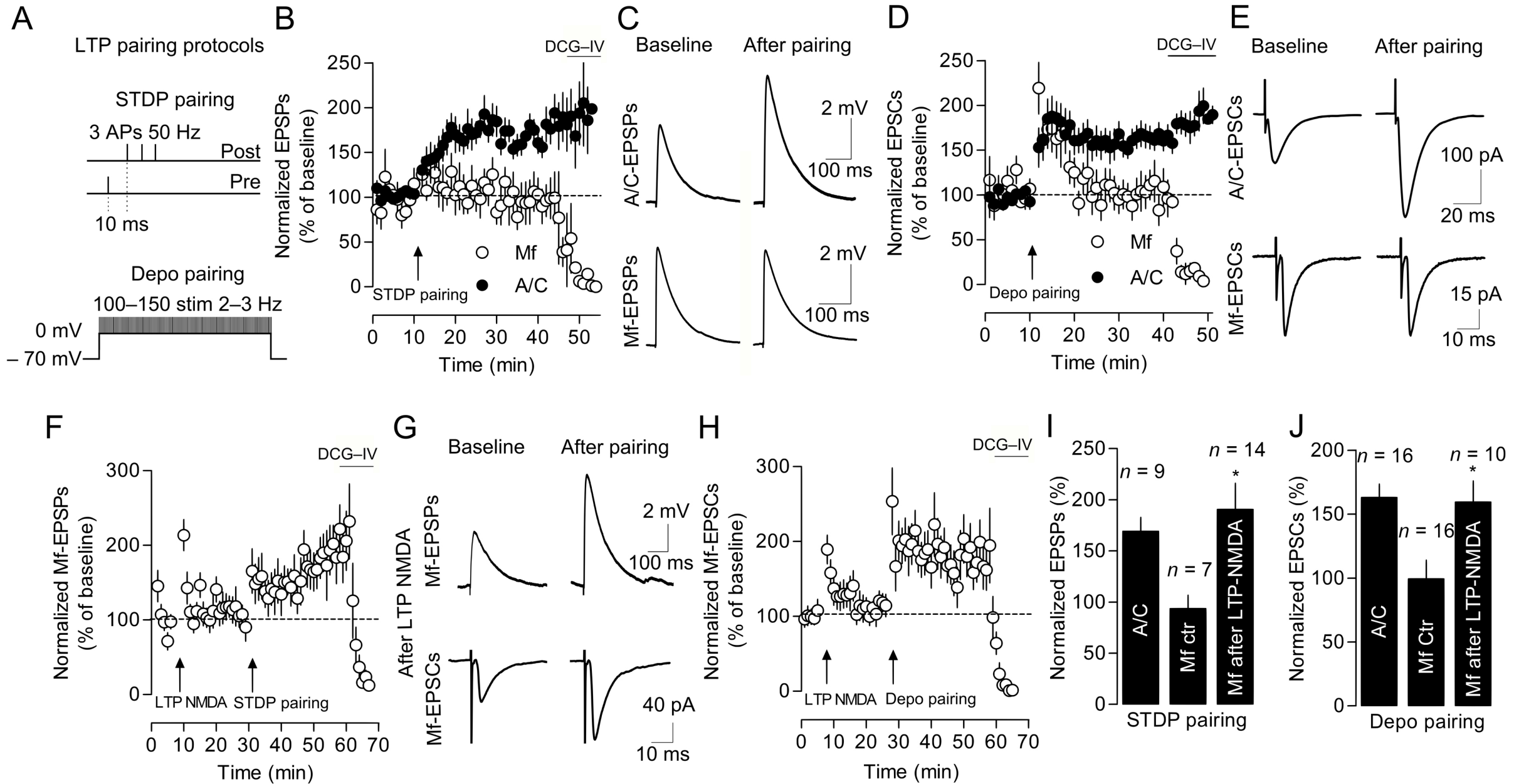


Figure 2

