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Asymmetrical Synaptic Cooperation between Cortical and Thalamic Inputs to the Amygdale

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Fear conditioning, a form of associative learning is thought to involve the induction of an associative long-term potentiation of cortical and thalamic inputs to the lateral amygdala. Here, we show that stimulation of the thalamic input can reinforce a transient form of plasticity (E-LTP) induced by weak stimulation of the cortical inputs. This synaptic cooperation occurs within a time window of 30 min, suggesting that synaptic integration at amygdala synapses can occur within large time windows. Interestingly, we found that synaptic cooperation is not symmetrical. Reinforcement of a thalamic E-LTP by subsequent cortical stimulation is only observed within a shorter time window. We found that activation of endocannabinoid CB1 receptors is involved in the time restriction of thalamic and cortical synaptic cooperation in an activity-dependent manner. Our results support the hypothesis that synaptic cooperation can underlie associative learning and that synaptic tagging and capture is a general mechanism in synaptic plasticity.
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INTRODUCTION

Learning from aversive events is a trait conserved across species as it is crucial for survival. However, generalizing responses to fear is nonadaptive and underlies several behavioral disorders (LeDoux, 2003). In auditory fear conditioning, a paradigm of Pavlovian associative learning, an emotionally neutral conditioned stimulus (CS) is paired with an aversive one (US), leading to an enhancement of the response to the CS. Plasticity in the amygdala, particularly in the lateral nucleus of the amygdala (LA), is critical for the long-term memory formation of conditioned fear (Johansen *et al*, 2011). Association between the auditory thalamic and auditory cortex input projections (CS) and the nociceptive input (US) induce a Hebbian long-term potentiation (LTP), leading to a persistent synaptic enhancement in thalamic and cortical inputs (Maren, 2005). Similar to what has been described in other brain areas, LTP induction in cortical and thalamic inputs to LA pyramidal neurons displays input specificity and involves calcium-dependent activation of CaMKII, MAPK and PKA (Huang and Kandel, 2007; Schafe *et al*, 2000). In addition, the persistence of LTP involves *de novo* protein synthesis (Schafe *et al*, 2000; Schafe and LeDoux, 2000) and postsynaptic AMPA receptor insertion (Rumpel *et al*, 2005). However, different forms of LTP have been described in thalamic LA and cortical LA synapses, with both inputs displaying postsynaptic and presynaptic forms of LTP depending on the exact afferent stimulation protocol and the degree of postsynaptic depolarization (Fourcaudot *et al*, 2009; Huang and Kandel, 1998; Shin *et al*, 2010). In the case of coincident activation of thalamic and cortical inputs, a heterosynaptic NMDA-dependent form of LTP is induced and expressed through presynaptic mechanisms (Humeau *et al*, 2003). Interestingly, postsynaptic induction of thalamic LTP can suppress thalamic presynaptic LTP via the CB1 endocannabinoid (eCB) receptors (Shin *et al*, 2010). How these different forms of thalamic and cortical LTP are orchestrated and the cellular mechanisms involved in the maintenance of thalamic and cortical association are not clear.

The maintenance of LTP also depends on past and future neuronal activity, which can be independent of the neuronal activity that occurs during the induction of LTP (Redondo and Morris, 2011). This concept has emerged from several reports showing that homosynaptic and heterosynaptic activity can modulate the maintenance of the long-lasting, protein synthesis-dependent forms of LTP (Fonseca *et al*, 2006b; Fonseca *et al*, 2006a). The induction of a long-lasting form of LTP (late-phase, L-LTP) in one set of synapses can stabilize a transient form of LTP (early-phase; E-LTP) in a second independent set of synapses by providing plasticity-related proteins (PRPs) that will be captured by 'tagged' synapses (Frey and Morris, 1998a). LTP maintenance is achieved by an interaction between input-specific 'synaptic tags', set by LTP induction, and the capture of PRPs synthesized in the soma or local dendritic domains. These are independent processes and can occur separately in time (Fonseca *et al*, 2004; Govindarajan *et al*, 2011; Redondo *et al*, 2010). Thus, according to the synaptic tagging and

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capture hypothesis, the maintenance of synaptic plasticity is a function of neuronal network activity and not only of the input stimulated at a given time. This conceptual framework provides a cellular mechanism that enables synapses to cooperate by local sharing of PRPs and neuronal networks to integrate neuronal activity processed continuously.

Here, we address the question whether synaptic tagging and capture is also involved in the amygdala thalamic LA and cortical LA association. We analyzed the ability of heterosynaptic cortical or thalamic L-LTP induction to convert an E-LTP induced at thalamic and cortical inputs into L-LTP. By altering the time window between thalamic and cortical input activation, we analyzed the temporal dynamics of this cooperative interaction. The demonstration of this cooperative reinforcement between cortical and thalamic inputs has a tremendous impact on the conceptual framework of associative fear learning, as it provides a cellular mechanism for continuous integration of information at amygdala synapses.

MATERIALS AND METHODS

Coronal brain slices (350 μm) containing the lateral amygdala nuclei were prepared from male Sprawgue–Dawley rats (3–4 weeks old) using a vibrotome (Leica, VT1200S). All procedures were approved by the Portuguese Veterinary Organization. The cutting ACSF was saturated with 95%O_2/5%CO_2 and contained (in mM) NaCl 126, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgCl_2$ 3, $CaCl_2$ 2 and Glucose 25. Slices were maintained in ACSF at 32 °C for at least 1 h before being transferred to a recording submersion chamber, and were perfused continuously (1.5–2 ml/min) with recording ACSF at 32 °C. The recording ACSF was saturated with 95%O_2/5%CO_2 and contained (in mM) NaCl 126, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $MgCl_2$ 2, $CaCl_2$ 2.5, Glucose 25. Whole-cell current-clamp recordings from pyramidal neurons were obtained with glass electrodes (7–10 MΩ; Harvard apparatus, UK), containing (in mM) K-gluconate 120, KCl 10, HEPES 15, Mg-ATP 3, Tris-GTP 0.3 Na-phosphocreatine 15 and creatine kinase 20 U/ml (adjusted to 7.25 pH with KOH, 290 mOsm). Putative pyramidal cells were selected by assessing the firing properties in response to steps of current (Figure 1b). Only cells that had a resting potential of less than –60 mV without holding current were taken further into the recordings. Neurons were kept at −70 to −75 mV with a holding current below −0.25 nA. Series resistance was monitored throughout the experiment and ranged from 30 to 40 MΩ; changes exceeding 20% of the series resistance determined the end of the recording. Stimulating electrodes (Science Products, GmbH, Germany) were placed on afferent fibers from the internal capsule (thalamic input) and from the external capsule (cortical input, Figure 1a). Pathway independence was checked by applying two pulses with a 50-ms interval to either the thalamic or cortical input and confirming the absence of crossed pair-pulse facilitation (PPF). Stimulus intensities were set to evoke 50% of the maximal excitory postsynaptic potential (EPSP) amplitude and LTP was induced after recording a stable baseline of EPSPs for 20 min. The test pulse frequency for each individual pathway was 0.033 Hz, except in the experiments where a third pathway was recorded as a control pathway in which the test pulse frequency for each individual pathway was 0.022 Hz. L-LTP was induced with a strong tetanic stimulation (25 pulses at a frequency of 100 Hz, repeated five times with an interval of 3 s), whereas transient LTP (E-LTP) was induced with a weak tetanic stimulation (25 pulses at a frequency of 100 Hz, repeated two times at an interval of 3 s). The following drugs were dissolved in DMSO and diluted to achieve the final concentration: Anisomycin (Sigma) 50 μM (in 0.02% DMSO), Verapamil (Sigma) 50 μM (in 0.01% DMSO), UBP302 (Sigma) 1 μM (in 0.01% DMSO), AM281 (Sigma) 0,5 μM (in 0.01% DMSO), Rapamycin (Tocris) 1 μM (in 0.01% DMSO), (RS)-α-Methyl-4-carboxyphenylglycine (MCPG; Tocris) 200 μM (in 0.01% DMSO). For the control experiments, only DMSO (0.02 or 0.01%) was added to the ACSF. AP-5 (Sigma) was dissolved in water and diluted in ACSF to achieve a final concentration of 50 μM. Electrophysiological data were collected using a RK-400 amplifier (Bio-Logic, France) filtered at 1 kHz and digitized at 10 kHz using a Lab-PCI-6014 data acquisition board (National Instruments, Austin, TX) and stored on a PC. Offline data analysis was performed using a customized LabView-program (National Instruments). As a measure for synaptic strength, the initial slope of the evoked EPSPs was calculated and expressed as percent changes from the baseline mean. Error bars denote SEM values. For the analysis, LTP values were averaged over 10-min data bins at three time windows, T1 = 20–30 min, T2 = 50–60 min, and T3 = 100–110 min. LTP decay was calculated by (T1 − T3)/T1 × 100. For the statistical analysis, control experiments of similar experimental design were pooled together. PPF was obtained by stimulation of the cortical or thalamic input fibers with two stimuli using an interstimulus interval of 50 ms. PPF values were obtained by dividing the slope of the second pulse by the slope of the first pulse. PPF changes were calculated as percentage changes from baseline mean at 3, 7 and 55 min after LTP induction. To test for group differences between LTP values across conditions, a repeated-measures ANOVA was performed with a Fisher *post hoc* test (Statistica StatSoft, Tulsa, OK).

RESULTS

Transient and Long-Lasting Forms of LTP can be Induced in both Cortical and Thalamic Input Projections to LA Nucleus

Previous studies showed that long-lasting forms of plasticity can be induced by pairing protocols both at the cortical and thalamic input afferents (Bauer *et al*, 2002). Initially, we set out to determine the stimulation protocol to induce both transient (E-LTP) and long-lasting forms of plasticity (L-LTP) at cortical and thalamic inputs. Whole-cell current-clamp recordings, showing spike frequency adaptation to steps of depolarizing current injection, were obtained from pyramidal neurons in the LA (Figure 1a and b). Stimulation of afferent fibers from the internal capsule (thalamic input), or from the external capsule (cortical input), evoked EPSPs of similar amplitude and slope. After a 20-min baseline recording, LTP was induced by a tetanic stimulation of the cortical or thalamic input with either a weak stimulation protocol (W–25 pulses at 100 Hz repeated two times, with a

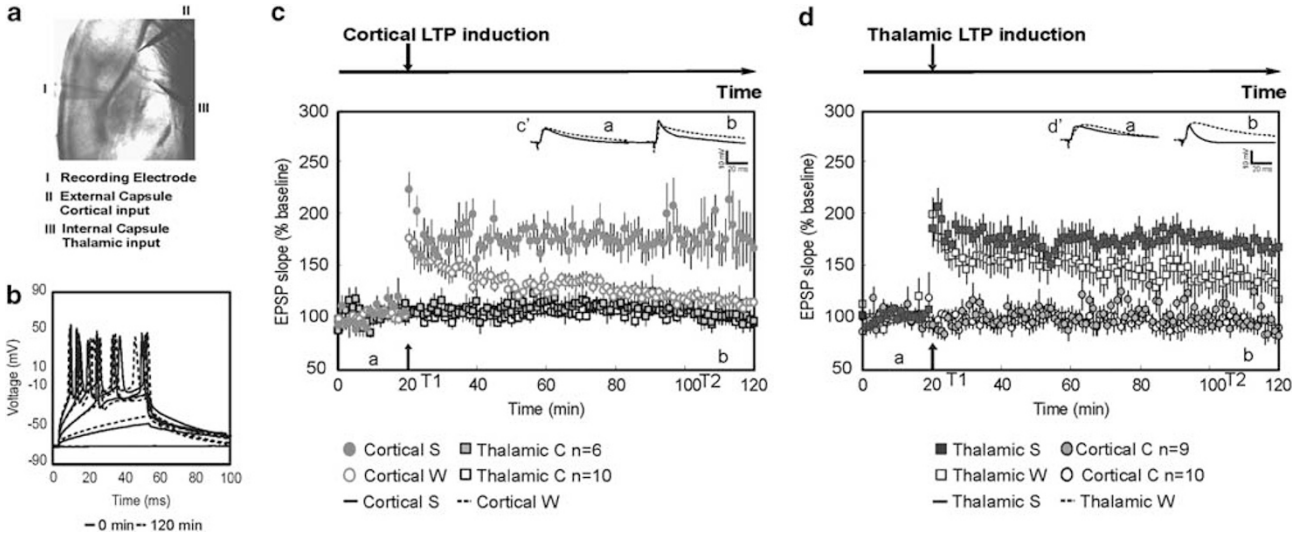


Figure 1 Transient and persistent forms of long-term potentiation (LTP) are induced by weak and strong stimulation of the cortical or thalamic input projections. (a) Positioning of the stimulating electrodes (II-cortical III-thalamic) and the recording electrode (I). (b) Voltage responses of a lateral nucleus of the amygdale (LA) pyramidal neuron cell in response to steps of depolarizing current injections. (c) Strong stimulation of the cortical input induced a maintained form of LTP ((cortical S) 171 ± 12.9%, $n = 6$), whereas weak stimulation of the cortical input results in a transient LTP ((cortical W) 117 ± 6.21%, $n = 10$). No change was observed in the basal synaptic transmission (thalamic control). (c′) Average EPSPs traces (average of three consecutive individual traces) for cortical S and cortical W, before (a) and after LTP induction (b). (d) Similar experiment as in C in the thalamic input (thalamic S 170 ± 7.6%, $n = 9$; thalamic W 132 ± 18.4%, $n = 10$). No change was observed in the basal synaptic transmission (cortical control). (d′) Average EPSPs traces for thalamic S and thalamic W, before (a) and after LTP induction (b). ± SEM. *n*, number of slices.

3-s interval) or a strong stimulation protocol (S–25 pulses at 100 Hz repeated five times, with a 3-s interval). Similar to what has been described in hippocampal Shaffer collateral to CA1 synapses, weak stimulation of the cortical or thalamic input resulted in a transient form of plasticity (early-phase LTP; E-LTP) that decayed to baseline values within the 2 h of recording. Conversely, strong stimulation of the cortical or thalamic input resulted in a L-LTP that was maintained throughout the duration of the recording. At the end of the recording, LTP induced by a weak stimulation was significantly lower than the LTP induced by a strong stimulation (Figure 1c and d). The non-stimulated inputs (thalamic and cortical, respectively) were used as control pathways showing no decrement in synaptic transmission throughout the duration of the recording.

Previous studies using pharmacological manipulations of NMDA receptors and voltage-gated calcium channels (VGCC) reported different effects in cortical and thalamic LTP inductions (Bauer *et al*, 2002). To further characterize the LTP induced by strong tetanic stimulation, we directly accessed the role of NMDA receptor and VGCC activation in the thalamic and cortical L-LTP. The application of AP-5 (50 μM), an NMDA receptor antagonist, blocked cortical L-LTP with only a mild impairment in the thalamic L-LTP (Figure 2a and b). Conversely, Verapamil (50 μM) application had the opposite effect, blocking preferentially the thalamic L-LTP (Figure 2a and b). LTP induced by strong cortical stimulation in AP-5-treated slices was significantly lower than that in Verapamil-treated slices and controls (Figure 2c), whereas LTP induced by thalamic strong stimulation was significantly lower in Verapamil-treated slices (Figure 2d). In all experiments, the non-stimulated inputs (thalamic and cortical, respectively, Figure 2a and b) were used as control pathways and were not affected in all tested conditions (open symbols). These results suggest that cortical L-LTP induction is strongly dependent on NMDA receptor activation, and thus relies on different cellular mechanisms than the thalamic L-LTP, the induction of which involves both NMDA receptor and VGCC activation.

LTP in the Thalamic Input has a Presynaptic Component Blocked by Inhibition of the GluR5 Kainate Receptors

Although it is generally accepted that the thalamic LA and cortical LA LTP can be induced and expressed postsynaptically, recent reports have shown that LTP induced at the cortical and thalamic inputs can also be expressed presynaptically (Huang and Kandel, 1998; Shin *et al*, 2010). In addition, the activation of L-type VGCC has been implicated in the induction of a presynaptic form of LTP (Fourcaudot *et al*, 2009). As we observed a strong dependence on VGCC activation in the induction of the thalamic L-LTP, it is conceivable that this form of long-lasting LTP has a presynaptic expression mechanism. This is of utmost relevance to us as synaptic cooperation is achieved by tagging activated synapses followed by the capture of postsynaptic PRPs. To test this, we measured the PPF ratio before and after LTP induction. Although changes in PPF ratio can exceptionally be induced by postsynaptic mechanisms (Wang and Kelly, 1997), it is generally the case that changes in PPF reflect a modulation of the presynaptic release probability (Shin *et al*, 2010; Tsvetkov *et al*, 2002). We found that LTP induction did not change PPF ratio (%baseline) in cortical inputs but significantly reduced the PPF ratio in thalamic inputs, a reduction that was still evident 1 h after LTP induction (Figure 2e). These results suggest that induction of LTP in the thalamic input had a presynaptic expression mechanism that was not observed in cortical L-LTP induction. As glutamate (GluR)5 kainate

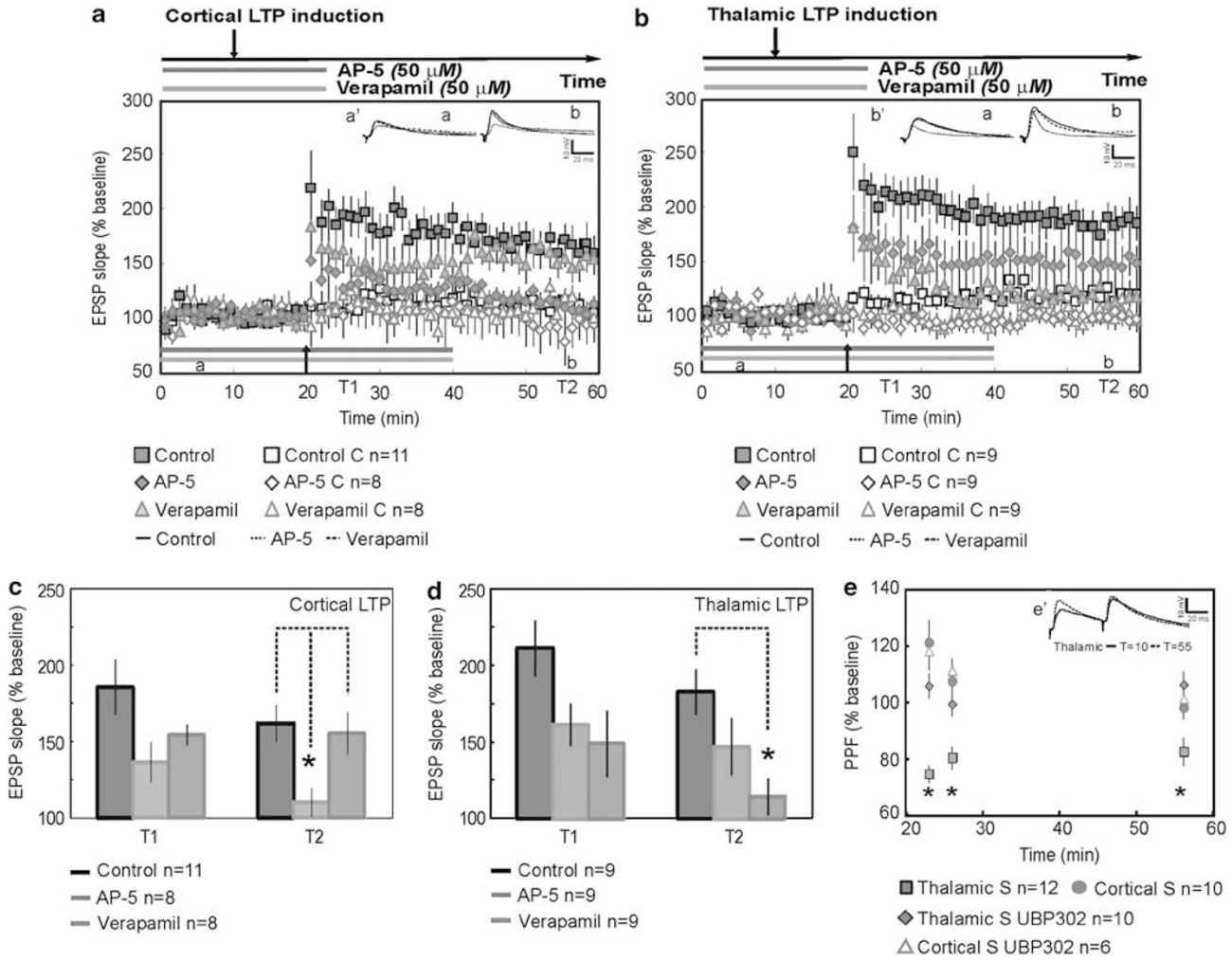


Figure 2 Inhibition of NMDA receptors and voltage-gated calcium channels (VGCC) differentially block cortical and thalamic L-LTP. (a) Application of AP-5 (50 μM◇) blocks cortical L-LTP induction, whereas application of Verapamil (50 μMΔ) results in a transient and nonsignificant decrease in long-term potentiation (LTP) (T1 cortical 185 ± 15.5%, $n=11$; cortical (AP-5) 137 ± 14.2%, $n=8$; cortical (Verapamil) 155 ± 6.7%, $n=8$; T2 cortical 162 ± 12.5%, $n=11$; cortical (AP-5) 110 ± 9.3%, $n=8$; cortical (Verapamil) 154 ± 13.7%, $n=8$). No change was observed in basal synaptic transmission (thalamic C□ ◇Δ). (a′) Average cortical excitory postsynaptic potentials (EPSPs) in control, AP-5 and Verapamil-treated slices, before (a) and after LTP induction (b). (b) Application of Verapamil (50 μMΔ) blocks thalamic L-LTP, whereas application of AP-5 (50 μM◇) results in nonsignificant decrease in LTP (T1 thalamic 212 ± 18%, $n=9$; thalamic (AP-5) 161 ± 13.7%, $n=9$; thalamic (Verapamil) 150 ± 22%, $n=9$; T2 thalamic 183 ± 14.8%, $n=9$; thalamic (AP-5) 147 ± 18.5%, $n=9$; thalamic (Verapamil) 114 ± 11.7%, $n=9$). No change was observed in the basal synaptic transmission (cortical C□ ◇Δ). (b′) Average thalamic EPSPs traces in control, AP-5 and Verapamil-treated slices, before (a) and after LTP induction (b). (c) Summary of LTP experiments at cortical–amygdala synapses showing the LTP decay for the two windows analyzed (T1 = 20–30 min and T2 = 50–60 min); ANOVA-repeated measures (T1 $F(2.24)=2.75$, $P=0.08$; T2 $F(2.24)=5.02$, $P=0.01$; Fisher's *post hoc* test cortical/cortical (AP-5), $P<0.01$; cortical/cortical (Verapamil), $P=0.7$; cortical (AP-5)/cortical (Verapamil), $P=0.02$; $^*P<0.05$. (d) Summary of LTP experiments in thalamic–amygdala synapses; ANOVA-repeated measures (T1 $F(2.24)=2.62$, $P=0.09$; T2 $F(2.24)=4.62$, $P=0.02$; Fisher's *post hoc* test thalamic/thalamic (AP-5), $P<0.01$; thalamic/thalamic (Verapamil), $P=0.11$; thalamic (AP-5)/thalamic (Verapamil), $P=0.19$, $^*P<0.01$). (e) Thalamic L-LTP induction (□) significantly reduces paired-pulse facilitation (PPF %baseline), whereas cortical L-LTP induction does not alter PPF (O). Application of UBP302 (1 μM◇) blocks the decrease in PPF observed upon thalamic LTP induction while has no impact in cortical PPF (Δ); ANOVA-repeated measures (T = 23 min, $F(3.34)=15.7$, $P<0.01$; T = 26 min, $F(3.34)=9.33$, $P<0.01$; T = 56 min, $F(3.34)=4.46$, $P<0.01$; Fisher's *post hoc* test thalamic/cortical $P<0.01$; thalamic/thalamic (UBP302), $P<0.01$; cortical/cortical (UBP302), $P>0.05$, for all time windows analyzed $^*P<0.01$. (e′) Average EPSPs traces for thalamic pair stimulation ± SEM. *n,* number of slices.

(KA) receptor activation has been implicated in the induction of presynaptic forms of thalamic LTP associated with a decrease in the PPF (Shin *et al*, 2010), to corroborate our results we tested the effect of inhibiting KA receptors in the thalamic LTP induction. We found that application of UBP302 (1 μM), an inhibitor of KA receptors, during the stimulation of the thalamic input blocked LTP induction (Supplementary Figure S1A and B). Conversely, application of UBP302 (1 μM), during the stimulation of the cortical input, had no impact in LTP induction (Supplementary Figure S1C and D). Analysis of the percentage change in PPF (%baseline) showed that UBP302 application blocked the decrease in PPF induced by thalamic L-LTP induction in control slices (Figure 2e) but had no impact in the cortical PPF (Figure 2e). These observations suggest that, unlike cortical L-LTP induction, strong stimulation of the thalamic input induces a form of L-LTP that relies on a presynaptic expression mechanism.

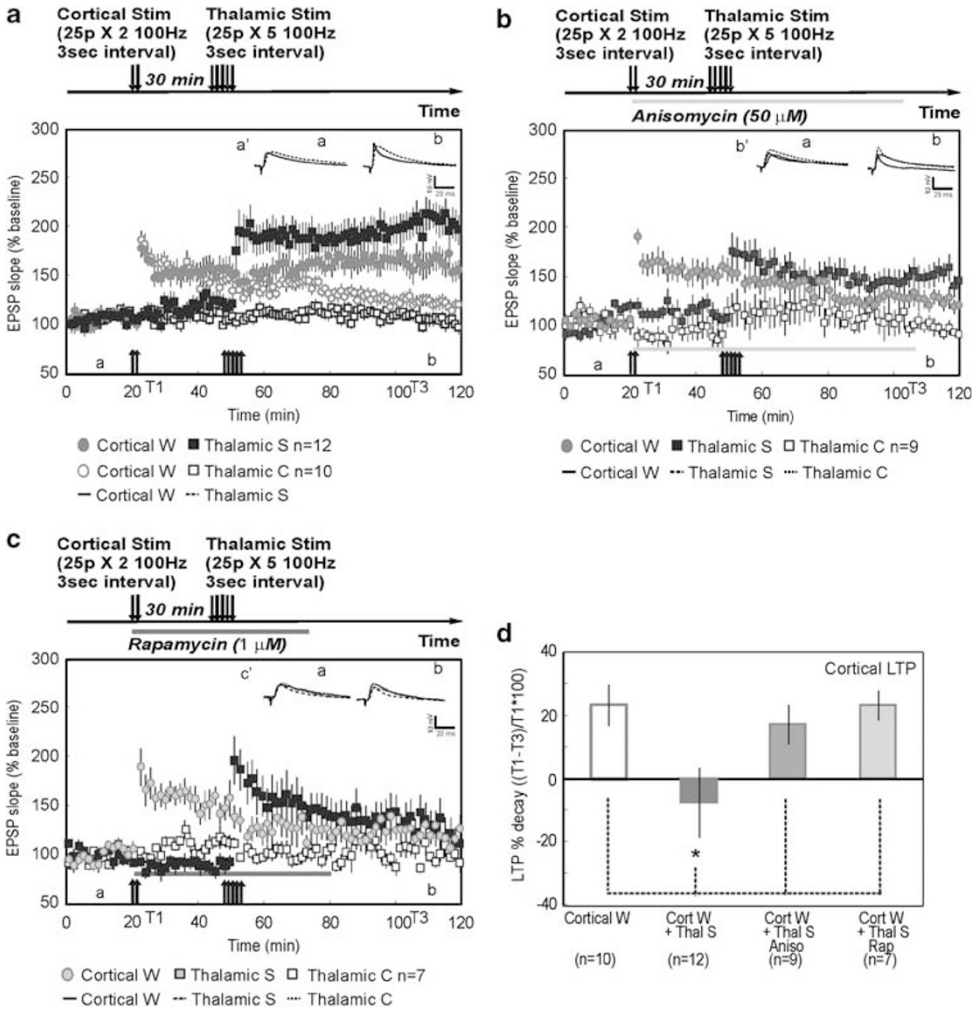
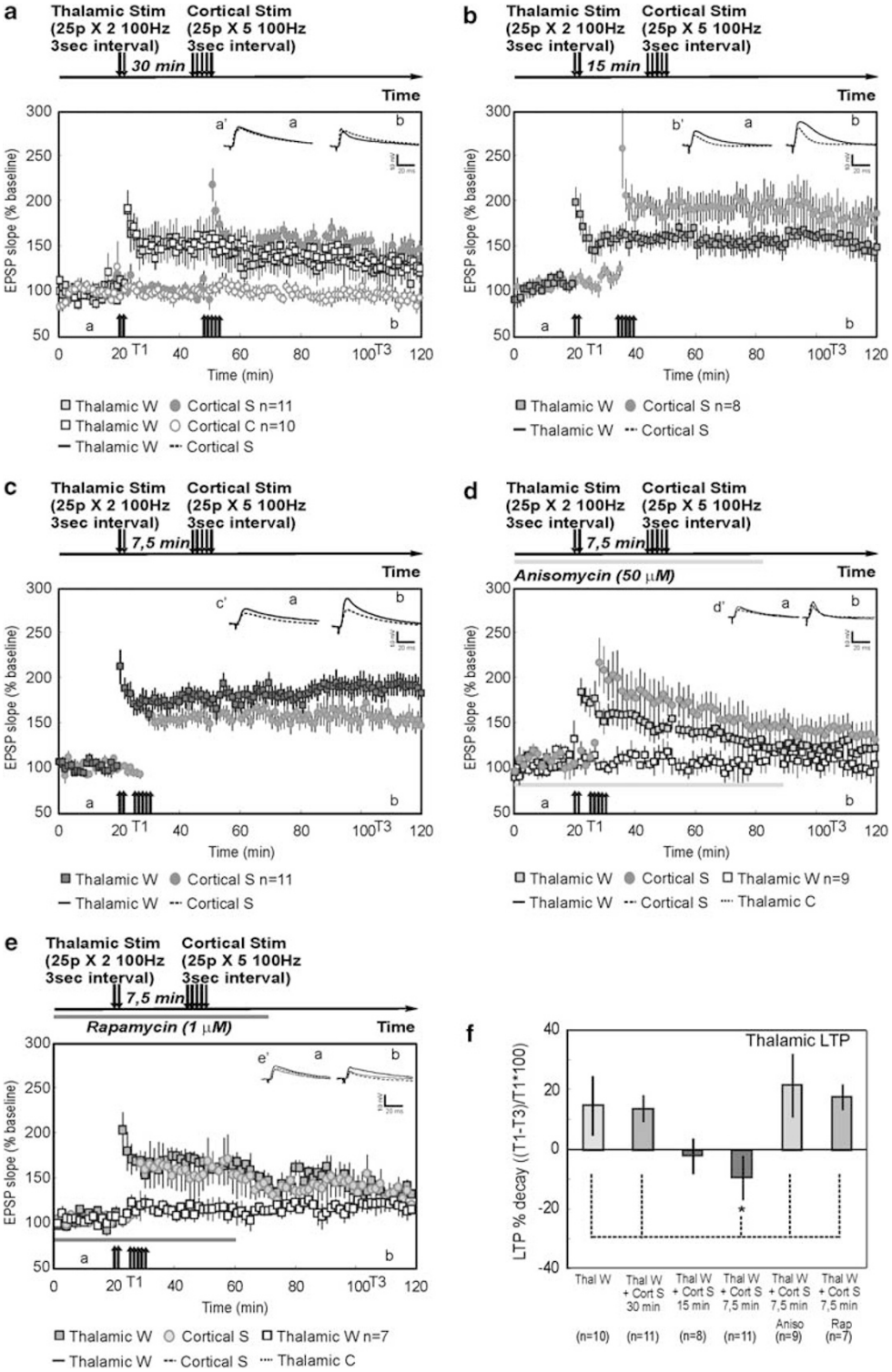


Figure 3 Cortical inputs can cooperate with thalamic inputs. (a) Induction of L-LTP by strong stimulation of the thalamic input (S□) is able to convert the E-LTP into L-LTP of the cortical input; (cortical W○) 117 ± 6.21%, $n = 10$; same data as in Figure 1c; cortical (W○) + thalamic S 154 ± 11.5%, $n = 12$). No change was observed in the basal synaptic transmission (thalamic control□). (a′) Average excitory postsynaptic potentials (EPSPs) traces for cortical W and thalamic S, before (a) and after LTP induction (b). (b) Application of Anisomycin (50 μM) blocks L-LTP induced by strong thalamic stimulation (S□) and the conversion of E-LTP into L-LTP of the cortical input (W○; cortical W + thalamic S + Anisomycin 125 ± 7.5%, $n = 9$). Anisomycin was applied starting after weak thalamic stimulation and washout 1 h after strong cortical stimulation (total duration of application was 90 min). No change was observed in the basal synaptic transmission (thalamic control□). (b′) Average EPSPs traces for cortical W, thalamic S and thalamic C, before (a) and after LTP induction (b). (c) Similarly, application of Rapamycin (1 μM) blocked the thalamic L-LTP induction (S□) and the conversion of E-LTP into L-LTP of the cortical input (W○; cortical W + thalamic S + Rapamycin 127 ± 10%, $n = 7$). No change was observed in the basal synaptic transmission (thalamic control□). (c′) Average EPSPs traces for cortical W, thalamic S and thalamic control c, before (a) and after LTP induction (b). (d) Summary plot showing the cortical LTP decay for the time windows analyzed, (T1 − T3)/T1 × 100. LTP decay is significantly lower for the condition in which cortical W stimulation is followed by thalamic S stimulation in control slices (cortical W 23.3 ± 6.3%, $n = 10$; cortical W + thalamic S −7.48 ± 11.2%, $n = 12$; cortical W + thalamic S + Anisomycin 17.2 ± 6.2%, $n = 9$; cortical W + thalamic S + Rapamycin 23.4 ± 5%, $n = 7$; ANOVA-repeated measures $F(3.37) = 3.3$, $P = 0.03$; Fisher's *post hoc* test cortical W/cortical W + thalamic S, $P = 0.01$; cortical W + thalamic S/cortical W + thalamic S + Anisomycin, $P = 0.03$; cortical W + thalamic S/cortical W + thalamic S + Rapamycin, $P = 0.02$, $^*P < 0.05$ ± SEM. *n,* number of slices.

Synaptic Cooperation between Cortical and Thalamic Inputs has Different Time Constraints

As mentioned previously, the synaptic tagging and capture hypothesis proposes a cellular mechanism that enables the association of events separated in time (Fonseca *et al*, 2004; Frey and Morris, 1998a; Redondo *et al*, 2010). To test whether cortical and thalamic inputs cooperate, we stimulated the cortical inputs with a weak stimulation protocol and assessed whether subsequent strong stimulation of the thalamic input is sufficient to convert the transient cortical LTP (E-LTP) into a long-lasting LTP (L-LTP). This experimental design was similar to the one used in hippocampal-tagging experiments to analyze heterosynaptic two-pathway interactions in a weak-before-strong configuration (Frey and Morris, 1998b). The strong thalamic stimulation was the trigger for PRP synthesis; hence, we called this configuration thalamic-to-cortical cooperation (experimental design depicted on the top of Figure 3a). We found that a transient form of LTP (E-LTP), induced by weak stimulation of the cortical input, was converted into a long-lasting form of LTP (L-LTP) by the subsequent induction of L-LTP in the thalamic input (Figure 3a). Using a large time interval of 30 min between cortical and thalamic input stimulations, we were able to induce thalamic-to-cortical cooperation. As thalamic-to-

cortical cooperation is based on PRPs sharing between tagged synapses, application of a protein synthesis inhibitor blocked synaptic cooperation. Anisomycin (50 μM) application blocked the induction of L-LTP in the thalamic input as well as the conversion of the E-LTP into L-LTP in the cortical input (Figure 3b). In this experiment, a third stimulation electrode was placed in the internal capsule allowing us to record a third input (thalamic control). Anisomycin application had no effect on baseline transmission or the viability of the slice (Figure 3b, open symbols). Similarly, application of Rapamycin (1 μM), an mTOR-dependent protein synthesis inhibitor (Cammalleri *et al*, 2003; Connor *et al*, 2011), blocked the induction of L-LTP in the thalamic input as well as the thalamic-to-cortical cooperation. No effect was seen in the control input (thalamic control) throughout the recording. Analysis of



LTP decay showed that E-LTP, induced by weak cortical stimulation, decayed significantly less if followed by subsequent thalamic L-LTP induction (Figure 3c). These results indicate that cortical and thalamic inputs can cooperate within relatively large time windows.

We then looked at the ability of cortical L-LTP induction to stabilize a transient form of LTP induced by weak stimulation of the thalamic input, a cortical-to-thalamic cooperation setting. Interestingly, we found that using the same time interval as before (30 min), the E-LTP induced in the thalamic input was not converted into an L-LTP by the subsequent cortical L-LTP induction (Figure 4a). The absence of cortical-to-thalamic cooperation using the same time interval as the thalamic-to-cortical cooperation opened two possibilities. One possibility is that the induction of LTP in the cortical input cannot provide the PRPs necessary for thalamic LTP maintenance because of distinct expression mechanisms. A second possibility is that the activity of the 'synaptic tag' induced by the thalamic E-LTP induction had a shorter duration. Previous studies have reported a decrease of the ability of the 'synaptic tag' to capture PRPs with time but within a relative large time window of about 1 h (Frey and Morris, 1998b; Redondo *et al*, 2010; Fonseca, 2012). To test whether the time interval between E-LTP induction in thalamic synapses and the L-LTP induction in cortical synapses had an impact on cortical-to-thalamic cooperation, we repeated the experiment described above but reduced the time interval to 15 and 7.5 min. We found that reducing the time interval between weak thalamic stimulation and strong cortical stimulation to 15 min led to an increase in the stabilization of the thalamic LTP (Figure 4b). Further reduction of the time interval between thalamic and cortical stimulations to 7.5 min significantly increased the cortical-to-thalamic cooperation (Figure 4c). As before, application of a protein synthesis inhibitor, Anisomycin (50 μM) or Rapamycin (1 μM), blocked the synaptic cooperation between thalamic and cortical inputs. In this case, and because the time interval between thalamic and cortical stimulations was markedly reduced, to maintain the duration of protein synthesis inhibition, Anisomycin (50 μM) or Rapamycin (1 μM) was applied during baseline recording. Inhibition of protein synthesis blocked the cortical L-LTP induction as well as the cortical-to-thalamic cooperation (Figure 4d and e). No effect was observed in the control pathway (thalamic control) throughout the recording (Figure 4d and e, open symbols). Analysis of LTP decay showed that cortical-to-thalamic synaptic cooperation was induced when weak thalamic stimulation was followed by strong cortical stimulation within a time interval of 7.5 min (Figure 4f). These results indicate that cooperation between cortical and thalamic inputs is bidirectional but asymmetrical in the time domain.

Inhibition of the CB1 eCB Receptor can Extend the Time Window of Thalamic–Cortical Cooperation in an Activity-Dependent Manner

Our results indicate that the time window of cortical-to-thalamic synaptic cooperation is shorter than thalamic-to-cortical cooperation, suggesting that the thalamic 'synaptic tag' activity to capture PRPs decays faster than the cortical 'synaptic tag'. As the cortical and thalamic LTP had different sensitivities to inhibitors of NMDA receptors and VGC channels, it is conceivable that different cellular mechanisms are involved in the induction of cortical and thalamic LTP conveying different properties to the 'synaptic tag', and therefore conveying different time intervals for cooperation. On the other hand, because L-LTP induced by a strong thalamic stimulation had a presynaptic expression mechanism, another possibility was that the 'synaptic tag' set by the induction of a presynaptic LTP had a shorter time window for cooperation. To distinguish between these two possibilities, we addressed the role of NMDA receptors and VGCC in the induction of the transient thalamic LTP. The transient form of LTP induced by weak thalamic stimulation was associated with the setting of the 'synaptic tag' and thus determined its duration and ability to capture PRPs. We found that NMDA receptor inhibition, by AP-5 application, had a strong impact on E-LTP (Supplementary Figure S2A), whereas inhibition of VGCC, by Verapamil application, had no effect on LTP values (Supplementary Figure S2B). Co-application of Verapamil with AP-5 did not

Figure 4 Thalamic input cooperation with cortical input operates within a shorter time interval. (a) Induction of L-LTP by strong stimulation of the cortical input (SO) 30 min after weak stimulation of the thalamic input (W□) did not induce cortical-to-thalamic cooperation (thalamic (W□) 132 ± 18.4%, $n = 10$; same data as in Figure 1c; thalamic (W□) + cortical S130 ± 11%, $n = 11$). No change was observed in the basal synaptic transmission (cortical control ○). (a′) Average excitory postsynaptic potentials (EPSPs) traces for thalamic W and cortical S, before (a) and after LTP induction (b). (b) Reduction of the time interval between strong stimulation of the cortical input (SO) and weak stimulation of the thalamic input (W□) to half (15 min) resulted in partial stabilization of the LTP expressed in the thalamic input (thalamic W + cortical S, 152 ± 9.55%, $n = 8$). (b′) Average EPSPs traces for thalamic W and cortical S, before (a) and after LTP induction (b). (c) Further reduction of the interval between strong stimulation of the cortical input (SO) and weak stimulation of the thalamic input (W□) to one-fourth of the initial time window (7.5 min) led to cortical-to-thalamic synaptic cooperation (thalamic W + cortical S 182 ± 10%, $n = 11$). (c′) Average EPSPs traces for thalamic W and cortical S, before (a) and after LTP induction (b). (d) Application of Anisomycin (50 μM) blocked cortical L-LTP (SO) and cortical-to-thalamic cooperation (thalamic W(□); thalamic W + cortical S + Anisomycin, 115.8 ± 14.7%, $n = 9$). Anisomycin was applied starting at the baseline and washout after strong cortical stimulation (total of 90 min). No change was observed in the basal synaptic transmission (thalamic control□). (d′) Average EPSPs traces for thalamic W, cortical S and thalamic C, before (a) and after LTP induction (b). (e) Similarly, application of Rapamycin (1 μM) blocked cortical L-LTP (SO) and cortical-to-thalamic cooperation (thalamic W(□); thalamic W + cortical S + Rapamycin, 138 ± 6%, $n = 7$). No change was observed in the basal synaptic transmission (thalamic control□). (e′) Average EPSPs traces for thalamic W, cortical S and thalamic C, before (a) and after LTP induction (b). (f) Summary plot showing the thalamic LTP decay for the time windows analyzed (T1 − T3)/T1 × 100; thalamic W 14.8 ± 9.9%, $n = 10$; thalamic W + cortical S (30 min), 13.8 ± 4.25%, $n = 11$; thalamic W + cortical S (15 min), − 2.25 ± 5.9%, $n = 8$; thalamic W + cortical S (7.5 min), − 9.71 ± 7.21%, $n = 11$; thalamic W + cortical S + Anisomycin (7.5 min), 21.6 ± 10.4%, $n = 9$; thalamic W + cortical S + Rapamycin (7.5 min), 17.8 ± 4%, $n = 7$; ANOVA-repeated measures $F(5.50) = 2.75$, $P = 0.03$, Fisher's *post hoc* test thalamic W + cortical S (7.5 min)/thalamic W, $P = 0.02$; thalamic W + cortical S (7.5 min)/thalamic W + cortical S (30 min), $P = 0.02$; thalamic W + cortical S (7.5 min)/thalamic W + cortical S (15 min), $P = 0.49$; thalamic W + cortical S (7.5 min)/thalamic W + cortical S + Anisomycin (7.5 min), $P < 0.01$; thalamic W + cortical S (7.5 min)/thalamic W + cortical S + Rapamycin (7.5 min), $P = 0.01$; $^*P < 0.05$ ± SEM. *n,* number of slices.

further reduce LTP as compared with AP-5-treated slices (Supplementary Figure S2A). These results showed that the thalamic E-LTP induction is dependent on NMDA receptor activation and not VGCC activation, suggesting a postsynaptic induction mechanism. Consistent with this, we found that inhibition of KA receptors with UBP302 had no impact on LTP induced by weak thalamic stimulation (Supplementary Figure S2B). Analysis of LTP values in all experimental conditions showed that application of AP-5 or the co-application of AP-5 and Verapamil significantly reduced LTP values at the end of the recording (Supplementary Figure S2C). These results suggest that the transient form of thalamic LTP had a similar induction mechanism as cortical L-LTP, and thus cannot explain the differences observed in the time interval for cooperation. We also analyzed the PPF changes induced by the thalamic E-LTP induction and the cortical-to-thalamic cooperation. We found that weak thalamic stimulation did not change PPF (%baseline), but induction of cooperation led to a significant reduction in the thalamic PPF (Supplementary Figure S2D). These results suggest that cortical-to-thalamic synaptic cooperation induces a presynaptic form of LTP that might add up with the postsynaptic LTP induced by the weak thalamic stimulation.

Previously, we had observed that synaptic activity can modulate the maintenance of LTP (Fonseca *et al*, 2006a; Fonseca, 2012). To test whether synaptic activation is involved in the time restriction of the cortical-to-thalamic cooperation, we repeated the experiment described before, in which the weak thalamic stimulation is separated from the strong cortical stimulation by 30 min, but suspending the thalamic synaptic activation between thalamic and cortical stimulations. In this configuration, that we call no-test pulse cooperation, the thalamic test pulse stimulation is presented only during 5 min after weak LTP induction and resumed after cortical L-LTP induction. Suspending synaptic activation had no impact in the decay of the weak thalamic LTP (Figure 5a) but was sufficient to restore the conversion of the thalamic E-LTP into L-LTP by the strong cortical stimulation (Figure 5a). This suggests that the time restriction of the cortical-to-thalamic cooperation is activity dependent.

As activation of CB1 receptors is implicated in the suppression of presynaptic thalamic LTP induction (Shin *et al*, 2010), one possibility is that activation of CB1 receptors, in an activity-dependent manner, suppresses the presynaptic thalamic LTP induction and blocks the cooperation. To test this, we induced cortical-to-thalamic cooperation with a 30-min interval between the thalamic weak stimulation and the strong cortical stimulation and applied AM281 (0.5 μM), an inhibitor of the CB1 receptors, during the interval between stimulations. We found that inhibition of CB1 receptors extends the time window for cortical-to-thalamic cooperation (Figure 5b), similar to what we observed when synaptic activation was suspended. eCBs can be released in the amygdala through the activation of metabotropic mGluRs (Azad *et al*, 2004; Varma *et al*, 2001); hence, one possibility is that synaptic activation triggers eCB release through mGluR activation. To test this, we repeated the experiment described above and inhibited mGluR activation by applying (RS)-α-MCPG (200 μM) during the time interval between weak thalamic stimulation and cortical strong stimulation (30 min). Blockade of mGluR receptors by MCPG also extended the time window for cortical-to-thalamic cooperation (Figure 5c). As NMDA receptor activation can also modulate the decay of LTP similar to synaptic activation (Fonseca, 2012), we tested whether APV application (50 μM) during the time interval between weak thalamic LTP induction and strong cortical LTP induction could also extend the cortical-to-thalamic cooperation. In this case, the application of APV was restricted to 20 min starting after weak thalamic LTP induction to avoid interfering with the induction of cortical L-LTP. We observed that blockade of NMDA receptors did not extend the cortical-to-thalamic cooperation (Figure 5d), suggesting that NMDA receptors are not involved in the restriction of the thalamic cooperation. Analysis of LTP decay showed that suspending synaptic activation, AM281 application or MCPG activation alone were not able to convert the E-LTP induced by weak thalamic stimulation into an L-LTP, but all these experimental conditions extended the time window for cortical-to-thalamic cooperation (Figure 5e). These results indicate that the eCB signaling, presumably through activation of mGluRs, limit the time window of cortical-to-thalamic cooperation.

DISCUSSION

We addressed the heterosynaptic interactions between cortical and thalamic afferents to projection neurons of the lateral amygdala, a circuitry necessary for the formation of fear-conditioning memories. We found that weak tetanic stimulation of the cortical amygdala input or the thalamic amygdala input led to the induction of a transient form of LTP that returns to baseline values within the duration of the recording. Conversely, strong tetanic stimulation of either the cortical or thalamic input led to the induction of a persistent form of LTP that was maintained throughout the recording. We have considerably extended the duration of the whole-cell current-clamp recordings to 2 h, which allowed us to address the cellular and molecular mechanisms involved in the maintenance of long-lasting forms of LTP (traditionally designated by late-phase LTP).

We found that LTP, induced by strong tetanic stimulation of the thalamic afferents to LA, was mainly dependent on the activation of L-type VGCC with a partial sensitivity to NMDA receptor inhibition. Moreover, we found that strong thalamic LTP resulted in a decrease in PPF ratio, suggesting a presynaptic expression mechanism (McKernan and Shinnick-Gallagher, 1997; Shin *et al*, 2010). The finding that strong thalamic LTP was sensitive to inhibition of GluR5 KA receptors (KA-GluR5) by application of UBP302 corroborated a presynaptic expression mechanism and is consistent with previous studies (Shin *et al*, 2010; Cho *et al*, 2012). Conversely, we found that cortical LTP, induced by strong tetanic stimulation of the cortical inputs, was dependent on NMDA receptor activation but not on VGCC, and its induction did not result in a decrease of PPF ratio. Moreover, cortical LTP was not sensitive to UBP302 application, which indicates that cortical LTP, under our experimental conditions, is expressed postsynaptically. We did not observe NMDA or VGCC activation contributing to basal synaptic transmission in thalamic and cortical inputs

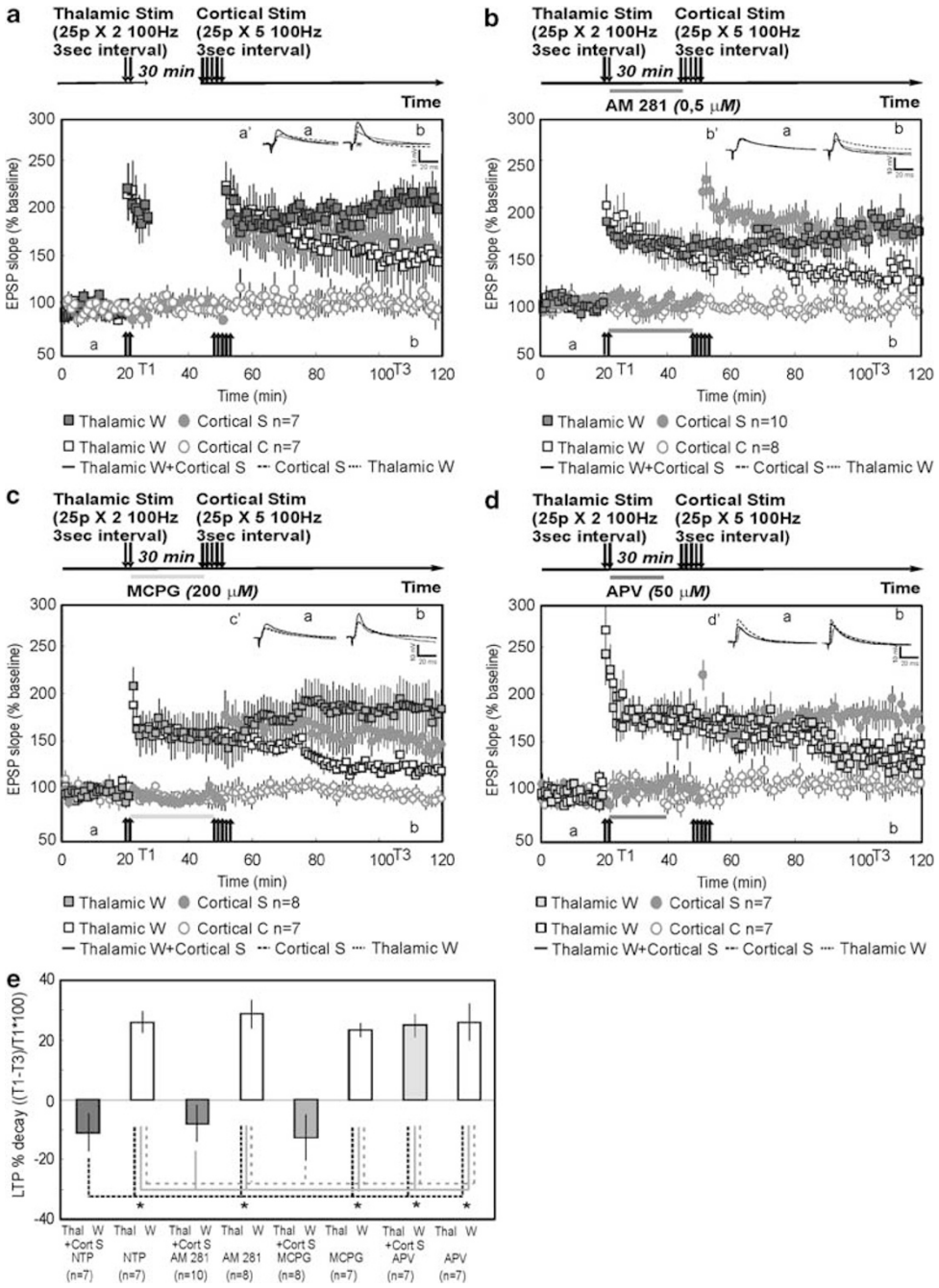


Figure 5 Cortical-to-thalamic synaptic cooperation can be extended by inhibition of the endocannabinoid (eCB) signaling in an activity-dependent manner. (a) Suspending synaptic activation after thalamic weak long-term potentiation (LTP) induction was sufficient to extend the window of cortical-to-thalamic cooperation to 30 min. Induction of L-LTP by strong stimulation of the cortical input was able to convert the E-LTP into L-LTP in the thalamic input if thalamic activation was suspended during the interval between weak thalamic and strong cortical stimulation (thalamic (W□) NTP 153 ± 26%, $n = 7$; thalamic (W□) + cortical (SO) NTP 216 ± 22%, $n = 7$). (a′) Average excitory postsynaptic potentials (EPSPs) traces for thalamic W + cortical S, cortical S and thalamic W, before (a) and after LTP induction (b). (b) Application of AM281 (0.5 μM) between weak thalamic stimulation and strong cortical stimulation also restored the cortical-to-thalamic cooperation (thalamic (W□) AM281 126 ± 10%, $n = 8$; thalamic (W□) AM281 + cortical (SO) 179 ± 11%, $n = 10$). (b′) Average EPSPs traces for thalamic W + cortical S, cortical S and thalamic (W), before (a) and after LTP induction (b). (c) Similarly, application of (RS)-α-Methyl-4-carboxyphenylglycine (MCPG; 200 μM) between weak thalamic stimulation and strong cortical stimulation also restores the cortical-to-thalamic cooperation (thalamic (W□) MCPG 190 ± 24%, $n = 8$; thalamic (W□) MCPG + cortical (SO) 125 ± 5%, $n = 7$). (c′) Average EPSPs traces for thalamic W + cortical S, cortical S and thalamic W, before (a) and after LTP induction (b). (d) Application of APV (50 μM), for 20 min, between weak thalamic stimulation and strong cortical stimulation does not restore the cortical-to-thalamic cooperation (thalamic (W□) APV145 ± 11%, $n = 7$; thalamic (W□) APV + cortical (SO) 137 ± 9%, $n = 7$). (d′) Average EPSPs traces for thalamic W + cortical S, cortical S and thalamic W, before (a) and after LTP induction (b). (e) Summary plot showing the thalamic LTP decay ((T1 − T3)/T1 × 100; thalamic W NTP 22 ± 4%, $n = 7$; thalamic W NTP + cortical S, − 11.1 ± 6%, $n = 7$; thalamic W AM281 28.6 ± 4.8%, $n = 8$; thalamic W AM281 + cortical S, − 7.9 ± 6.1%, $n = 10$; thalamic W MCPG 23 ± 2.5%, $n = 7$; thalamic W MCPG + cortical S, − 12 ± 7.6%, $n = 8$; thalamic W APV 26 ± 6%, $n = 7$; thalamic W APV + cortical S 25 ± 4%, $n = 7$; ANOVA-repeated measures $F(7,52) = 10.8$, $P < 0.01$, Fisher's *post hoc* test $^*P < 0.01$ for all conditions compared) ± SEM. *n*, number of slices; NTP, no-test pulse cooperation.

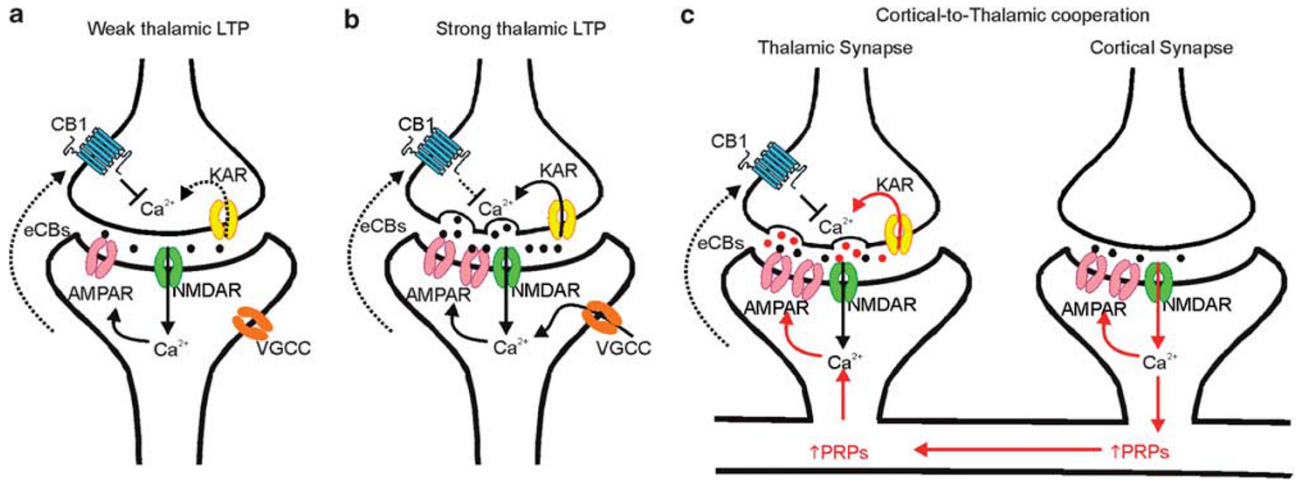


Figure 6 Potential mechanism for the cortical-to-thalamic cooperation. (a) Weak stimulation of the thalamic input induces a transient form of long-term potentiation (LTP), which is dependent on NMDA receptor activation and expressed postsynaptically. The expression of a presynaptic form of LTP is blocked by the retrograde signaling of the endocannabinoids (eCBs) via activation of presynaptic CB1 receptors. (b) Strong thalamic stimulation induces a long-lasting form of LTP that is expressed pre- and postsynaptically and that involves activation of postsynaptic NMDA receptors and voltage-gated calcium channels (VGCC) as well as presynaptic KA receptors. (c) In the setting of cortical-to-thalamic cooperation, strong stimulation of the cortical input induces a long-lasting LTP that induces an upregulation of PRPs that are captured at the cortical and thalamic tagged synapses. If cooperation is induced within a short time window (7.5 min) the inhibitory effect of CB1 receptor activation is not sufficient to block the cooperation and LTP in the thalamic input is reinforced. If strong cortical activation happens too late (30-min interval) the inhibitory effect of CB1 receptor activation is effective in blocking the presynaptic expression of LTP and therefore effective in blocking the cortical-to-thalamic cooperation.

as application of either AP-5 or Verapamil had no impact on baseline EPSP slope (Mahanty and Sah, 1999).

Interestingly, both pre- and postsynaptic forms of cortical LTP were reported in the literature, depending on the stimulation pattern used to induce LTP (Fourcaudot *et al*, 2009; Humeau *et al*, 2003; Shaban *et al*, 2006; Huang and Kandel, 1998; Tsvetkov *et al*, 2002). Presynaptic forms of cortical LTP are reliably induced by coincident activation of cortical and thalamic inputs and are dependent on presynaptic NMDA receptor activation and postsynaptic L-type VGC channels activation (Fourcaudot *et al*, 2009). Conversely, tetanic stimulation is associated with the induction of a postsynaptic form of LTP, which is dependent on the postsynaptic influx of Ca^{2+} and NMDA receptor activation (Huang and Kandel, 1998). Our results are consistent with the involvement of VGCC activation in the induction of presynaptic LTP and NMDA receptor activation in the induction of postsynaptic LTP. Further, the observation that cortical LTP, induced by strong tetanic stimulation, is not dependent on VGCC activation might explain why we did not observe any changes in PPF ratio (Fourcaudot *et al*, 2009). As inhibition was intact in our recordings as compared with several previous studies where EPSP and PPF recordings were performed in the presence of picrotoxin (a GABAa antagonist; Fourcaudot *et al*, 2009; Humeau *et al*, 2003; Shaban *et al*, 2006; Tsvetkov *et al*, 2002), it is possible that in our experimental conditions, the threshold for VGCC activation was increased. Consistent with this hypothesis, we found that weak thalamic stimulation led to the induction of a transient form of LTP that was dependent on NMDA receptor activation but not VGCC, with no change in the PPF ratio. In addition, inhibition of KA-GluR5 receptors did not block weak thalamic LTP, which supports a postsynaptic expression mechanism. Consistent with previous reports (Bauer *et al*, 2002), our results suggest that the recruitment of VGCC is associated with the strength of the stimulation, with weak thalamic stimulation leading to the induction of a postsynaptic NMDA receptor-dependent LTP, whereas strong thalamic stimulation recruits VGCC and KA-Glur5 receptors and leads to the induction of pre- and postsynaptic forms of LTP (Figure 6a and b). Our results also show that the threshold for LTP induction in the thalamic input is lower (Doyere *et al*, 2003), as cortical strong stimulation led to the induction of a postsynaptic, NMDA-dependent form of LTP but did not recruit VGCC. Our data do not exclude the possibility that other forms of LTP, namely presynaptic LTP, can be induced at the cortical input. However, we believe that the 100-Hz tetanic stimulation resembles the activation of the thalamic and cortical inputs during fear learning (Kwon and Choi, 2009), and therefore the LTP induced by the stimulation pattern used here adequately represents the change in synaptic strength induced by fear-conditioning learning. Furthermore, by changing the number of trains applied to the thalamic and cortical inputs during tetanic stimulation, we were able to induce transient and long-lasting forms of LTP, which robustly model short- and long-term memory formations.

Although the induction of homosynaptic forms of LTP in either the cortical or thalamic input to LA allows us to detail the cellular and molecular mechanisms involved in LTP induction and maintenance, it is clear that during fear-conditioning learning, both inputs are active and interact with each other (Doyere *et al*, 2003). Associated forms of plasticity were described previously and involve coincident activation of cortical and thalamic afferents (Humeau *et al*, 2003). We have demonstrated a different form of associative plasticity in which a transient form of homosynaptic LTP is

reinforced by subsequent activation of a heterosynaptic input. This associated plasticity occurs within a large time window and is dependent on the capture of PRPs by previously tagged synapses, leading to the expression of long-lasting forms of LTP in both activated inputs. We found that weak cortical stimulation led to the induction of a transient form of LTP, which can be converted into a long-lasting form of LTP by subsequent strong stimulation of the thalamic input. Similar to what has been described in hippocampal Schaffer collateral to CA1 synapses (Frey and Morris, 1998a; Frey and Morris, 1998b; Redondo *et al*, 2010), the thalamic-to-cortical synaptic cooperation is blocked by protein synthesis inhibition, strengthening the view that the sharing and capture of PRPs by the activated cortical and thalamic inputs underlies the conversion of the transient cortical LTP into a long-lasting LTP. We found that the cooperation between the cortical and thalamic inputs is bidirectional but the temporal constraints are asymmetrical. The conversion of a transient thalamic LTP into a long-lasting LTP can only be achieved if the subsequent strong stimulation of the cortical afferents is presented within a short time window (7.5 min). The cortical-to-thalamic cooperation is also dependent on *de novo* protein synthesis suggesting a similar underlying cellular mechanism, ie, the sharing and capture of PRPs by tagged cortical and thalamic synapses. Thus, we favor the hypothesis that the duration of the tag, ie, its activity to capture the PRPs, is shorter in weak thalamic stimulation as compared with weak cortical stimulation. Although the identity of the 'synaptic tag' remains elusive, recent studies suggest that the 'synaptic tag' should be seen as a temporary structural and/or functional state of the synapse, independent of the direction of the plastic change of the synapse and the molecular mechanisms involved in the maintenance of this plastic change (Fonseca, 2012; Redondo *et al*, 2010; Redondo and Morris, 2011). In this functional purview, any cellular mechanism that constrains the expression of synaptic plasticity, eg, from permissive to nonpermissive, is a component of the synaptic tag. We found that inhibition of eCB receptor CB1 led to an extension of the cortical-to-thalamic cooperation window, suggesting that activation of CB1 restricts synaptic cooperation. Our hypothesis is supported by previous findings, where the induction of a postsynaptic thalamic LTP, via activation of CB1 receptors, led to the suppression of a presynaptic thalamic LTP (Shin *et al*, 2010). Our observation that the reinforcement effect in thalamic LTP induced by synaptic cooperation led to the induction of a presynaptic thalamic LTP also supports this hypothesis (Figure 6c). Interestingly, we found that suspending synaptic activation or pharmacological blockade of mGluR also led to the extension of the cortical-to-thalamic cooperation window, suggesting that synaptic activation, presumably through activation of mGluR leads to the release of eCBs that acting on CB1 receptors restricts synaptic cooperation. This is supported by previous studies showing a modulation of eCBs release by mGluR activation (Varma *et al*, 2001; Azad *et al*, 2004). Our results do not suggest that CB1 receptor activation regulates the synthesis of PRPs but rather modulates the ability of thalamic synapse to capture PRPs or to express LTP. Although recent studies report a link between CB1 activation and the modulation of protein synthesis (Busquets-Garcia *et al*, 2013; Puighermanal *et al*, 2009), both show that CB1 receptor blockade downregulates the activity of the mTOR pathway, reducing protein translation. We observed that blockade of CB1 facilitates synaptic cooperation and as activation of CB1 receptors suppresses the induction of presynaptic thalamic LTP (Shin *et al*, 2010), our interpretation is that activity-dependent release of eCB restricts the ability of thalamic synapses to benefit from the PRPs synthesized upon cortical L-LTP induction.

What might be the significance of this thalamic and cortical synaptic cooperation? One possibility is that the association between cortical and thalamic projection is necessary for a discriminative form of fear learning. Although the activation of either the cortical or thalamic input is sufficient for fear-conditioning learning (Campeau and Davis, 1995; Kwon and Choi, 2009), in auditory discriminative fear learning, coactivation of both inputs might be necessary for discrimination (Antunes and Moita, 2010). This observation is consistent with a cooperative interaction between the cortical and thalamic inputs. What is then the functional consequence of this differential time window for the thalamic-to-cortical *vs* cortical-to-thalamic synaptic cooperation? One possibility is that restricting the time window of cortical-to-thalamic cooperation protects from generalizing fear responses. Consistent with this, increasing the expression of CREB in the direct thalamic LA input enhances fear learning and leads to generalization in discriminative fear-learning task (Han *et al*, 2008). If the reinforcement of the thalamic input leads to generalization of a fear response, then restricting the time window for cortical-to-thalamic cooperation would decrease generalized responses. Interestingly, there is evidence that eCBs releases, acting via CB1 receptors, modulate the expression of both generalized and cue-fear responses during fear conditioning (Patel and Hillard, 2006; Reich *et al*, 2008). The restricting effect of CB1 receptor activation on the cortical-to-thalamic cooperation could represent a cellular mechanism to avoid fear generalization.

Recently, several studies have shown that memories can interact with each other by means of synaptic tagging and capture (Almaguer-Melian *et al*, 2012; Ballarini *et al*, 2009; Moncada and Viola, 2007; Wang *et al*, 2010; Myskiw *et al*, 2013). All these studies show that exposure to novelty can upregulate the synthesis of PRPs, presumably through the release of dopamine, which are then captured at tagged synapses in a cooperative fashion. It remains to be addressed whether associative learning can be induced and/or modulated by synaptic tagging and capture. Altogether, we present compelling evidence that synaptic tagging and capture is a general cellular mechanism to integrate multiple stimuli over large time windows. Considering the similar properties between cellular forms of plasticity, such as LTP, and memory establishment, it is of utmost relevance to explore whether the processes of synaptic tagging and synaptic cooperation operate in discriminative fear learning.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)