Research

Noradrenergic stabilization of heterosynaptic LTP requires activation of Epac in the hippocampus

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Beta-adrenergic receptor (β-AR) activation by norepinephrine (NE) enhances memory and stabilizes long-term potentiation (LTP), a form of synaptic plasticity believed to underlie some forms of hippocampal memory. LTP can occur at multiple synaptic pathways as a result of strong stimulation to one pathway preceding milder stimulation of an adjacent, independent pathway. Synaptic tagging allows LTP to be transferred, or captured, at heterosynaptic pathways. Previous research has shown that β-AR activation promotes heterosynaptic LTP by engaging various signaling cascades. In particular, cyclic adenosine monophosphate (cAMP) activates cAMP-dependent protein kinase A (PKA) and guanine nucleotide exchange protein activated by cAMP (Epac), to enhance LTP. Epac activation can occlude subsequent induction of stable homosynaptic LTP after β-AR activation, but it is unclear whether Epac activation is required for heterosynaptic LTP following pairing of the natural transmitter, NE, with one 100 Hz train of stimulation ("NE-LTP"). Using electrophysiologic recordings of CA1 field excitatory postsynaptic potentials during stimulation of two independent synaptic pathways in murine hippocampal slices, we show that distinct inhibitors of Epac blocked stabilization of homo- and heterosynaptic NE-LTP. PKA inhibition also attenuated heterosynaptic transfer of NE-LTP, but only when a PKA inhibitor was applied during tetanization of a second, heterosynaptic pathway that was not treated with NE. Our data suggest that NE, paired with 100 Hz, activates Epac to stabilize homo- and heterosynaptic LTP. Epac may regulate the production of plasticity-related proteins and subsequent synaptic capture of NE-LTP at a heterosynaptic pathway. Epac activation under these conditions may enable behavioral experiences that engage noradrenergic inputs to hippocampal circuits to be transformed into stable long-term memories.

Norepinephrine (NE) is a neuromodulatory transmitter secreted in response to arousal and novelty (Aston-Jones and Bloom 1981; Sara and Segal 1991). Noradrenergic fibers project from the locus coeruleus to innervate the hippocampus, which expresses beta-adrenergic receptors (β-ARs) that bind NE (Hillman et al. 2005). Activation of β-ARs by NE engages signaling cascades that facilitate long-term neural plasticity (Stanton and Sarvey 1984; Harley et al. 1996; Katsuki et al. 1997; for review, see Nguyen and Gelinas 2018) and memory formation (Izquierdo et al. 1998; Straube et al. 2003; Lemon et al. 2009; for review, see O'Dell et al. 2015). Activation of β-ARs in area CA1 of the hippocampus, a brain structure critical for memory formation (Scoville and Milner 1957; Zola-Morgan et al. 1986; Eichenbaum 2000), facilitates activity-dependent increases in synaptic strength (Thomas et al. 1996; Gelinas and Nguyen 2005; for review, see O'Dell et al. 2015).

One type of hippocampal synaptic plasticity is long-term potentiation (LTP) (Bliss and Lømo 1973). LTP is believed to be a cellular mechanism for memory formation in the mammalian brain (Bliss and Collingridge 1993; Bourtchuladze et al. 1994; Ji et al. 2003a; Gelinas and Nguyen 2005; Whitlock et al. 2006; for review, see Martin et al. 2000), and it can be sustained by treating in vitro hippocampal slices with either a β-AR agonist, isoproterenol (ISO) (Thomas et al. 1996; Katsuki et al. 1997; Gelinas and Nguyen 2005), or with the natural β-AR ligand, NE (Katsuki et al. 1997; Hu et al. 2007; Maity et al. 2016; for review, see O'Dell et al. 2015). Furthermore, β-AR activation by ISO or NE boosts the endurance of LTP by activating signaling kinases to modulate translation initiation and increase the synthesis of specific proteins (Winder et al. 1999; Klann et al. 2004; Gelinas et al. 2007; Maity et al. 2015; for review, see O'Dell et al. 2015). In general, translation is critical for stabilizing LTP (Krug et al. 1984; Costa-Mattioli et al. 2009; Maity et al. 2015).

Heterosynaptic LTP occurs when synaptic activity at one group of synapses initiates cellular mechanisms that elicit synaptic potentiation at a second group of synapses converging on the same postsynaptic neurons. One potential cellular mechanism for heterosynaptic LTP is synaptic tagging (Frey and Morris 1997). According to this model, an LTP-inducing stimulus generates a local synaptic "tag" at one set of tetanized synapses. Tags function to capture plasticity-related proteins (PRPs) that are generated at a different group of synapses that had previously experienced strong stimulation. Normally, applying a modest LTP induction protocol (e.g., one train at 100 Hz) to a "homosynaptic" pathway induces decremental potentiation. However, eliciting persistent LTP with stronger stimulation at another convergent pathway will transfer LTP to the weakly stimulated pathway, leading to long-lasting potentiation at both pathways (Frey and Morris 1997; Sajikumar et al. 2007). Importantly, ISO-induced persistent homosynaptic LTP at one pathway can be captured at a second, heterosynaptic pathway (Connor et al. 2011). However, it is unclear whether the natural β-AR ligand, NE, can facilitate heterosynaptic capture of LTP.

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Article is online at http://www.learnmem.org/cgi/doi/10.1101/lm.048660.118.

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3′,5′-Cyclic adenosine monophosphate (cAMP) is a key second messenger that is strongly implicated in hippocampal LTP and memory consolidation. Stimulation of cAMP signaling in area CA1 is sufficient to initiate long-lasting synaptic potentiation (Frey et al. 1993). Inhibiting or mutating hippocampal cAMP-dependent protein kinase (PKA), which is activated by cAMP, impairs tetanus-induced heterosynaptic LTP (Young et al. 2006) and blocks hippocampal memory consolidation (Abel et al. 1997). ISO-induced heterosynaptic LTP requires PKA activation in murine area CA1 (Connor et al. 2011). Besides PKA, another target of cAMP is guanine exchange protein directly activated by cAMP (Epac). Epacs are expressed in the nervous system (Kawasaki et al. 1998) and they bind cAMP to activate a GTPase, Rap, in a PKA-independent fashion (de Rooij et al. 1998). In area CA1 of murine hippocampal slices, pharmacologic activation of Epac stabilizes homosynaptic LTP independent of PKA and it occludes subsequent expression of tetanus-induced homosynaptic LTP (Gelinas et al. 2008). However, it is unknown whether activation of Epac or PKA is required for stabilization of NE-mediated LTP.

In area CA1 of murine hippocampal slices, pairing bath application of NE with one train of 100 Hz stimulation elicits long-lasting homosynaptic LTP ("NE-LTP"; Maity et al. 2016). In the present study, we characterized the effects of NE on heterosynaptic LTP by using an in vitro, dual synaptic pathway stimulation protocol. In area CA1 of murine hippocampal slices, two independent populations of synapses contacting the same postsynaptic cells were monitored to assess the effects of prior induction of homosynaptic NE-LTP at one pathway (S1) on the subsequent induction of heterosynaptic LTP at a second pathway (S2). We asked two questions: First, can NE paired with 100 Hz stimulation in S1 elicit stable heterosynaptic LTP in S2? Second, if NE-LTP can be produced, does its stabilization require activation of Epac? Using specific pharmacological inhibitors of Epac and PKA, we show that long-lasting NE-LTP occurred mostly through a PKA-independent mechanism. Stabilization of homosynaptic and heterosynaptic NE-LTP required Epac activation during 100 Hz stimulation at S1 and S2. In contrast, PKA activation was required for heterosynaptic transfer of NE-LTP only during 100 Hz stimulation at S2, but not during paired 100 Hz and NE application at S1. Our results reveal a novel mechanistic dichotomy for heterosynaptic NE-LTP stabilization, involving possibly distinct contributions from Epac and PKA.

Results

Norepinephrine stabilizes heterosynaptic LTP

NE binds to β-ARs to facilitate potentiation at excitatory hippocampal synapses (Stanton and Sarvey 1984; Harley et al. 1996; Katsuki et al. 1997). When applied before, or during, a single 100 Hz tetanus that normally elicits decremental LTP, NE enables the expression of long-lasting homosynaptic LTP in area CA1 of mouse hippocampal slices ("NE-LTP": Maity et al. 2015, 2016). Pairing ISO, a β-AR agonist, with one train of 100 Hz (Gelinas and Nguyen 2005; Gelinas et al. 2007) or with low-frequency stimulation (Thomas et al. 1996) also induces long-lasting homosynaptic LTP ("ISO-LTP"). ISO-LTP can be evoked at a second heterosynaptic input in mouse area CA1, thus implicating a role for β-ARs in modulating synaptic tagging and capture of PRPs at these synapses (Connor et al. 2011), processes believed to enable synapse-specific transfer of LTP between activated pathways (Frey and Morris 1997). However, it is unclear whether NE, the natural ligand of adrenoreceptors, can stabilize heterosynaptic LTP in area CA1.

To test this idea, we first needed to establish the effects of one train of 100 Hz on field excitatory postsynaptic potentials (fEPSPs) in the Schaeffer collateral pathway of CA1. Upon stimulation of S1 (the homosynaptic pathway) and S2 (the heterosynaptic pathway) 30 min apart, LTP at S1 and S2 was not sustained (Fig. 1A,C 100 Hz columns). This finding is corroborated by previous research, in which a paired stimulation protocol, without any applied transmitter agonist, was insufficient for inducing long-lasting LTP (Connor et al. 2011). Thus, one train of 100 Hz applied to separate pathways did not elicit enduring LTP. Our results show that S1, having received only a single tetanus, did not promote subsequent capture of LTP by tags set later, at S2.

To determine if NE can stabilize heterosynaptic LTP, we treated slices with NE for 10 min prior to one train of 100 Hz at S1. NE

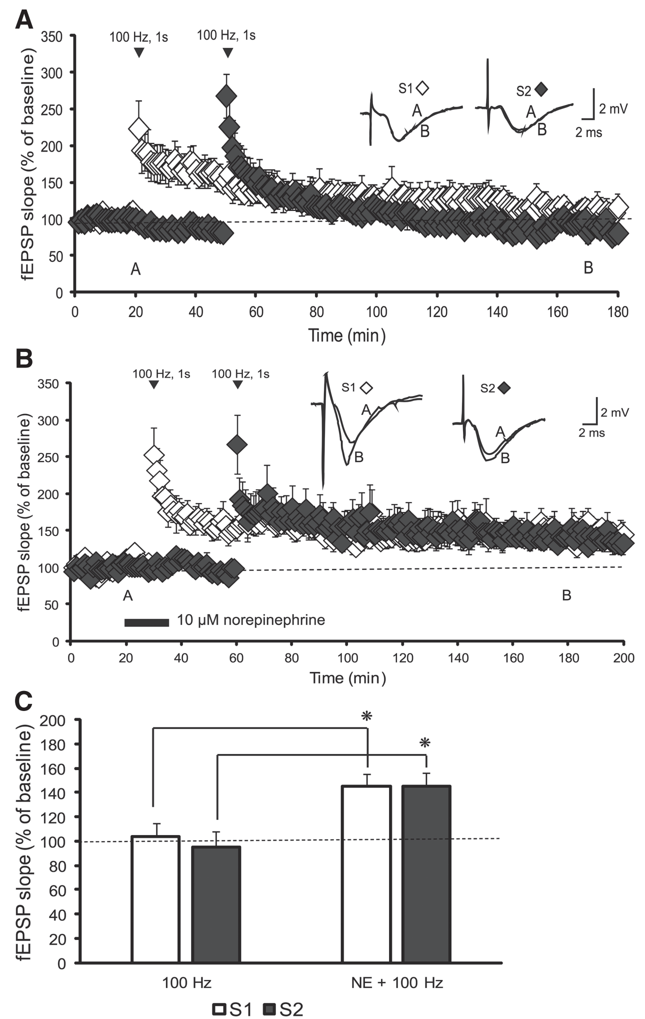

Figure 1. NE facilitates the expression of heterosynaptic LTP. (*A*) Stimulation (one train of 100 Hz for 1 sec) of the homosynaptic pathway (S1; open symbols) induced transient (<1 h) LTP which failed to promote heterosynaptic LTP upon administering an identical stimulus to a second synaptic pathway (S2; filled symbols) 30 min later ($n = 7$). Stimulation at 100 Hz alone did not persistently strengthen synapses, as fEPSP slopes at S1 and S2 returned to baseline (within 1 h). (*B*) NE application along with 100 Hz produced persistent homosynaptic LTP in S1 (NE-LTP) that was subsequently captured by 100 Hz stimulation alone at S2 ($n = 6$). Representative fEPSP traces were sampled 20 min after commencement of baseline recordings and 120 and 90 min after 100 Hz stimulation at S1 and S2, respectively. (*C*) Summary histogram comparing mean fEPSP slopes obtained 150 min after 100 Hz at S1 (white bars) and 120 min after 100 Hz at S2 (gray bars). (*) Specifies statistical significance ($P < 0.05$) between treatment groups. Results in C denote means ± SEM.

continued to perfuse the slices for 5 min post-100 Hz before wash-out. This protocol, but without NE, was administered to S2 30 min after S1 stimulation (Fig. 1B). Student's *t*-test comparisons between control- and NE-treated slices revealed significant differences in the stabilization of LTP, both homosynaptically ($P<0.05$; mean fEPSP slope in NE-treated slices was 145 ± 10% of baseline, which was significantly elevated relative to control slices, 104 ± 11% of baseline) and heterosynaptically ($P<0.05$; mean fEPSP slope was 145 ± 11% in NE-treated slices versus 95 ± 13% in controls; Fig. 1C "NE + 100 Hz"). Thus, NE-LTP generated at one synaptic pathway leads to long-lasting LTP at a second pathway.

Stabilization of heterosynaptic NE-LTP does not require PKA activation

Previous studies have revealed that PKA, a downstream target of β-AR activation, is a key signaling effector for synaptic plasticity and memory consolidation (Abel et al. 1997; Nayak et al. 1998; Duffy et al. 2001; Nguyen and Woo 2003; Woo et al. 2003; Abel and Nguyen 2008). A role for PKA has also been implicated in β-AR-induced LTP (Thomas et al. 1996) and in synaptic tagging during tetanus-induced late-LTP (Young et al. 2006; Connor et al. 2011). To determine if PKA activation is required for heterosynaptic NE-LTP, we applied an NE-LTP protocol to S1, consisting of one train of 100 Hz overlapping with bath application of NE in the presence of PKA inhibitors, KT5720 (KT) or PKI. Each inhibitor was bath-applied for 30 min and washed out prior to stimulation of S2 30 min after S1 stimulation. Addition of PKI (Fig. 2B; mean fEPSP slope was 142 ± 14% of baseline) or KT (Fig. 2C; mean fEPSP slope was 167 ± 18% of baseline) during 100 Hz at S1 failed to inhibit homosynaptic NE-LTP at S1 relative to inhibitor-free controls (Fig. 2A; mean fEPSP slope from controls was 151 ± 10% of baseline). Furthermore, neither KT nor PKI significantly impaired the endurance of heterosynaptic NE-LTP at S2 (Fig. 2B,C, mean slopes in S2 were: KT 150 ± 14% of baseline, PKI 139 ± 9% of baseline), as compared to controls (Fig. 2A).

When KT application was shifted to overlap with 100 Hz at S2 (Fig. 2D), the heterosynaptic pathway experienced decremental

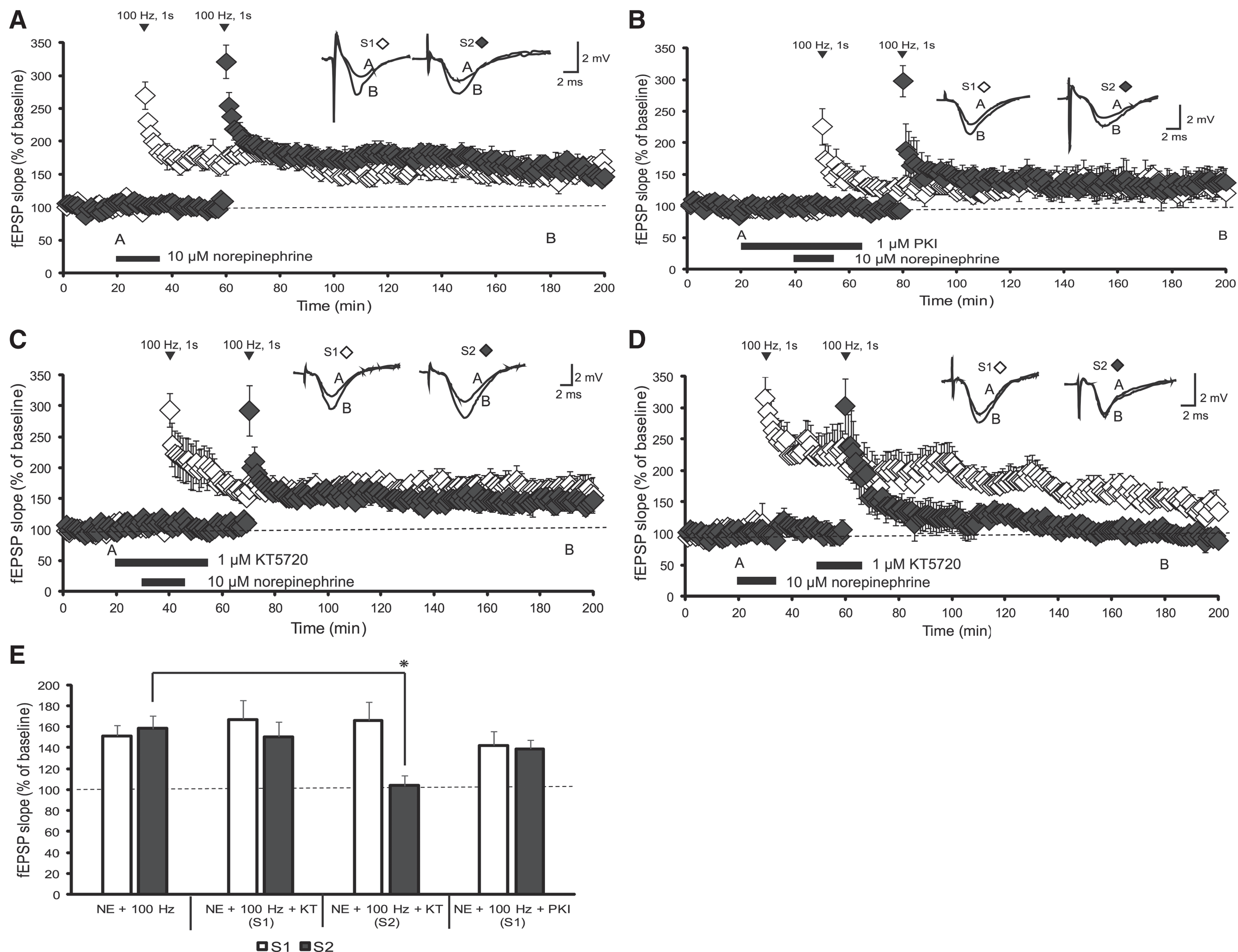

Figure 2. PKA activation is not required for homosynaptic NE-LTP but is required during 100 Hz stimulation at a heterosynaptic pathway for transfer of stable NE-LTP. (*A*) Both homosynaptic (open symbols) and heterosynaptic (filled symbols) NE-LTP were persistently expressed following coincident application of NE overlapping with 100 Hz stimulation to S1 ($n=6$). When overlapped with NE at S1, the PKA inhibitors, (*B*) protein kinase A inhibitor peptide (PKI; $n=5$), and (*C*) KT5720 (KT; $n=7$) did not impair homosynaptic or heterosynaptic LTP. (*D*) Shifting KT application to overlap with 100 Hz at S2 induced decremental heterosynaptic LTP ($n=6$). Representative traces were sampled 20 min after commencement of baseline recordings and 120 and 90 min after 100 Hz at S1 and S2, respectively. (*E*) Summary histogram comparing mean fEPSP slopes obtained 150 min after 100 Hz at S1 (white bars) and 120 min after 100 Hz at S2 (gray bars). (*) Specifies statistical significance ($P<0.05$) between treatment groups. Results in *E* denote means ± SEM.

LTP, whereas S1 (which received prior 100 Hz + NE) successfully expressed NE-LTP (mean fEPSP slope was 166 ± 18% of baseline at S1 compared to controls and 104 ± 10% of baseline at S2). An ANOVA comparing fEPSP slopes of both PKA inhibitor treatments showed no significant differences between groups at S1 ($F_{(3,21)}$ = 0.53; $P > 0.05$); however, there was a difference at S2 ($F_{(3,21)}$ = 3.91; $P < 0.05$) (Fig. 2E). A subsequent Tukey–Kramer post-hoc test revealed that KT, when shifted to overlap with 100 Hz at S2, prevented heterosynaptic transfer of NE-LTP to S2 ($P < 0.001$). Thus, PKA inhibition failed to impair the stability of homo- and heterosynaptic LTP when applied during NE application at S1. In contrast, PKA inhibition during S2 (100 Hz alone) disrupted heterosynaptic transfer of NE-LTP to those "tagged" synapses. In our previous study (Maity et al. 2015), it was shown that NE + 100 Hz boosted translation of specific mRNAs (i.e., synthesis of PRPs) during homosynaptic NE-LTP. Taken together with our present data, we conclude that PKA activation is not required for synthesis of PRPs triggered by 100 Hz + NE treatment at S1.

Inhibiting Epac prevents expression of NE-LTP at tagged synapses

A second signaling molecule which cAMP directly activates is Epac. Several findings have implicated Epac activation in hippocampal memory and hippocampal LTP. For example, cAMP signaling modulates memory consolidation through Epac, independent of PKA (Ma et al. 2009). Furthermore, pharmacologic activation of Epac strengthens hippocampal synapses by inducing translation-dependent potentiation, which in turn, occluded subsequent induction of long-lasting ISO-LTP (Gelinas et al. 2008). However, the roles of Epac activation in heterosynaptic NE-LTP remain undefined. Particularly, it is unknown whether inhibition of Epac can affect heterosynaptic NE-LTP at hippocampal synapses.

To probe the roles of Epac activation on heterosynaptic NE-LTP, we first established that heterosynaptic NE-LTP could be reliably produced by S2 100 Hz, given 30 min after S1 100 Hz in the presence of NE. Mean fEPSP slopes of control slices (treated with NE at S1) were 142 ± 6% of baseline and 162 ± 11% of baseline at S1 and S2, respectively (Fig. 3A). We then applied NE + 100 Hz in the presence of an Epac inhibitor, brefeldin A (BFA). This treatment significantly impaired the stabilities of homosynaptic (Fig. 3B; mean fEPSP slope for S1 was 96 ± 5% of baseline) and heterosynaptic NE-LTP (mean fEPSP slope for S2 was 101 ± 7% of baseline). We also applied a more specific blocker of Epac, ESI-09 (ESI); this also significantly suppressed homosynaptic and heterosynaptic NE-LTP stability (Fig. 3C; mean fEPSP slopes were 103 ± 6% of baseline at S1 and 105 ± 7% at S2). Shifting the time of application of BFA, to overlap with 100 Hz at S2, prevented heterosynaptic transfer of NE-LTP from S1: only the homosynaptic (S1) pathway remained potentiated throughout the recording period (Fig. 3D; mean fEPSP slopes were 129 ± 4% of baseline at S1 compared to controls and 91 ± 10% of baseline at S2 compared to controls). ESI applied during S2 yielded similar results (Fig 3E; average fEPSP slopes were 142 ± 20% at S1 and 88 ± 2% at S2).

An ANOVA was performed to compare differences among pharmacological treatments and a control group. The test identified significant differences between groups homosynaptically ($F_{(4,23)} = 4.10$; $P < 0.05$) and heterosynaptically ($F_{(4,23)} = 14.07$; $P < 0.001$) (Fig. 3F). A subsequent Tukey–Kramer post-hoc test revealed that ESI and BFA prevented stabilization of homosynaptic (BFA: $P < 0.05$; ESI: $P < 0.05$) and heterosynaptic (BFA: $P < 0.001$; ESI: $P < 0.001$) NE-LTP. Additionally, relative to control slices treated only with NE and 100 Hz, applying ESI or BFA during S2 100 Hz impaired heterosynaptic, but not homosynaptic, LTP stabilization (BFA: $P < 0.001$; ESI: $P < 0.001$). Taken together, our data suggest that activation of Epac, but not of PKA, is required for production of plasticity-related components (proteins?) needed for heterosynaptic transfer of NE-LTP. In contrast, heterosynaptic transfer of NE-LTP to tagged synapses (S2) depends on the activation of Epac during S1 and S2 stimulation, and on PKA activation during S2 stimulation.

Discussion

NE promotes the formation, retrieval, and retention of hippocampus-dependent spatial and contextual memories, through activation of β-ARs (Sara et al. 1999; Ji et al. 2003a,b; Murchison et al. 2004). Additionally, β-ARs modulate hippocampal LTP through the activation of signaling mechanisms that include glutamatergic AMPA receptors, PKA, Epac, ERK, and translation initiation (for review, see O'Dell et al. 2015). Epac has been implicated in distinct forms of synaptic plasticity, including hippocampal homosynaptic LTP (Gelinas et al. 2008; Yang et al. 2012) and cerebellar homosynaptic LTP linked to motor learning (Gutierrez-Castellanos et al. 2017). However, to date, no study has probed the roles of Epac activation in heterosynaptic LTP.

The present study explored the roles of Epac in NE-mediated heterosynaptic plasticity in area CA1 of murine hippocampal slices. Unlike previous studies using the synthetic β-AR agonist, ISO, we have used here the physiological β-AR ligand, NE. We found that NE, when paired with one train of 100 Hz stimulation, facilitated stabilization of heterosynaptic LTP. Our findings are consistent with previous research that used ISO: Connor et al. (2011) found that ISO paired with one train of 100 Hz at S1 enabled subsequent capture of long-lasting LTP at S2. Interestingly, 5 Hz stimulation for 10 sec was used at S2 (Connor et al. 2011), and heterosynaptic LTP required activation of β-ARs, as it was blocked by a β-AR antagonist (Connor et al. 2011). Qian et al. (2012) showed that prolonged 5 Hz stimulation in mouse CA1 in the presence of ISO elicited homosynaptic LTP that required activation of β-2, but not β-1, adrenergic receptors. Our previous research has shown that homosynaptic NE-LTP after 100 Hz stimulation in CA1 was dependent on activation of β-1 and β-2 receptors, but did not require activation of α-ARs (Maity et al. 2016). Thus, the collective findings show that activation of β-ARs, either with ISO or with the natural transmitter NE (the present study), gates the stabilization of long-lasting synaptic potentiation when paired with low- or high-frequency stimulation.

Our data reveal a novel mechanistic dichotomy in the roles of PKA and Epac during heterosynaptic NE-LTP. We found that activation of Epac during S1 and S2 was required for homo- and heterosynaptic NE-LTP. In contrast, PKA activation was required only during 100 Hz stimulation at S2 (without applied NE at S2) for heterosynaptic capture of NE-LTP. These results are concordant with our earlier studies (Connor et al. 2011) in which a PKA inhibitor (PKI) applied during S1 tetanization (with ISO applied) had no effect on homo- or heterosynaptic ISO-LTP, but blocked heterosynaptic capture of ISO-LTP when it was applied during S2 stimulation at 5 Hz. Thus, PKA activation appears to be critical for synaptic tagging/capture at S2, and not for triggering the synthesis of PRPs during S1 tetanization paired with β-AR activation. Young et al. (2006) provided data from mouse CA1, using a strong multi-train tetanization protocol without β-AR activators to elicit homosynaptic late-LTP, to support the notion that PKA activation is needed for synaptic tagging (see also Park et al. 2014, for a presynaptic role of PKA in tagging during homosynaptic LTP). Connor et al. (2011) showed that protein synthesis during S2 tetanization (5 Hz, 10 sec) was not needed for heterosynaptic capture of ISO-LTP; such synthesis was shown instead to be required during S1 tetanization paired with ISO. It is noteworthy that generation

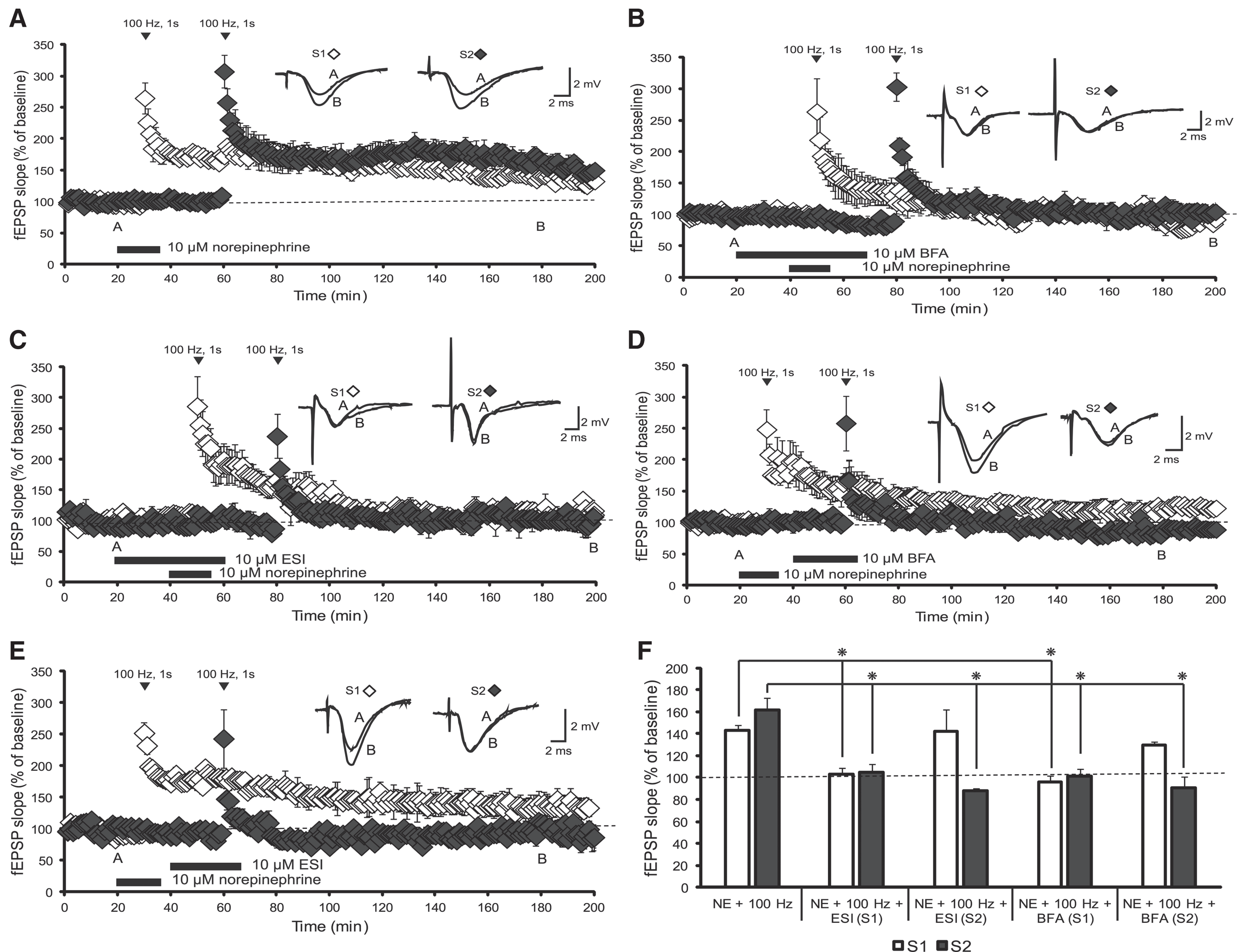

Figure 3. Epac activation is required for heterosynaptic transfer of NE-mediated LTP. (*A*) NE-LTP was elicited at S1 (open symbols) and subsequently captured at a second pathway (filled symbols) ($n = 6$). Treating hippocampal slices with the Epac inhibitors (*B*) brefeldin A (BFA) or (*C*) ESI-09 (ESI), as S1 received 100 Hz stimulation, reduced homosynaptic LTP stabilization in both pathways ($n = 5$). Delaying application of (*D*) BFA or (*E*) ESI to overlap with 100 Hz at S2, with 100 Hz and NE administered earlier to S1, prevented heterosynaptic transfer of NE-LTP ($n = 6$). Note that neither BFA nor ESI disrupted LTP maintenance at S1 when applied during 100 Hz at S2 (open symbols for S1, in *D* and *E*). Representative traces were sampled 20 min after commencement of baseline recordings and 120 and 90 min after 100 Hz at S1 and S2, respectively. (*F*) Summary histogram comparing average fEPSP slopes obtained 150 min after 100 Hz at S1 (white bars) and 120 min after 100 Hz at S2 (gray bars). * specifies statistical significance ($P < 0.05$) between treatment groups. Results in *F* denote means ± SEM.

of local synaptic tags does not require protein synthesis (Frey and Morris 1997). However, the process of local translation may serve as a tag, since its action allows for the generation of spatially restricted proteins that can modify active synapses to stabilize LTP. In general, the role of PKA may be limited to establishing synaptic tags (to mark activated synapses) following application of relatively modest stimulation (i.e., 1 train of 100 Hz) (Barco et al. 2002; Young et al. 2006). During heterosynaptic NE-LTP, PKA may mediate synaptic tagging (and/or subsequent capture of PRPs) at a heterosynaptic pathway after prior NE-LTP induction at a distinct pathway that triggers synthesis of PRPs. PKA's role seems less strongly linked to initiation of translation at S1 during paired application of NE and 100 Hz tetanization there. PKA itself may be a tag, since its local anchoring at hippocampal synapses plays critical roles in mediating homosynaptic LTP (Nie et al. 2007). Further research is needed to identify proteins phosphorylated by PKA during heterosynaptic NE-LTP.

Our present experiments highlight possible dual roles for Epac activation: recruitment of translation during S1 tetanization paired with NE, and subsequent capture of NE-LTP at S2. Occlusion experiments have demonstrated that ISO-LTP and Epac-induced potentiation (evoked by applying an Epac agonist) share similar underlying mechanisms: the latter occluded subsequent ISO-LTP (Gelinas et al. 2008). Pharmacologic activation of Epac increases hippocampal expression of phosphorylated extracellular signal-regulated protein kinase (ERK) (Gelinas et al. 2008), a key regulator of translation-dependent β-AR LTP (Gelinas et al. 2007; Connor et al. 2011). Phosphorylated ERK is a prime modulator of translation initiation in eukaryotic cells (for reviews, see Klann et al. 2004; Kelleher et al. 2008; Costa-Mattioli et al. 2009). Thus, one key role for Epac activation during NE-LTP may be recruitment of translation initiation, in conjunction with NE, which was paired with 100 Hz at S1. It is likely that Epac is assisting other factors in mediating translation recruitment. Either NE, or 100 Hz, alone is not enough to significantly boost translation in mouse CA1 (Maity et al. 2015). However, both stimuli applied together (NE + 100 Hz) can significantly boost the synthesis of specific proteins in murine CA1, concomitant with stabilization of homosynaptic

NE-LTP (Maity et al. 2015). We speculate that Epac activation may be required during this pairing in order to link downstream engagement of the ERK pathway with subsequent translation regulation needed for production of PRPs that are subsequently captured by S2. Indeed, inhibition of Epac activation during S2 tetanization in our present study prevented heterosynaptic capture of NE-LTP that was consolidated earlier at S1. Additionally, we emphasize that Epac activation by NE paired with 100 Hz at S1 is required, but not sufficient, for capture of NE-LTP at S2. In support of this, we showed that PKA activation was also required at S2 for heterosynaptic capture.

Additional support for the general notion that cAMP-mediated signaling is critical for the synthesis of PRPs comes from studies of synaptic tagging and LTP following rolipram-induced synaptic potentiation. By boosting cAMP levels in the hippocampus, rolipram converted unstable LTP to resilient LTP that required translation in rat hippocampal slices (Navakkode et al. 2004). Further research is needed to probe the exact actions of Epac during heterosynaptic NE-LTP. Can Epac act as a local tag, possibly by being localized at active synapses? There is a paucity of evidence to directly show Epac anchoring at hippocampal synapses, although there are data supporting a role for a PKA anchoring protein, AKAP150, in coordinating Epac-mediated phosphorylation (Nijholt et al. 2008). Are other signaling kinases (besides ERK) recruited by Epac activation to engage translation? Characterizing Epac's compartmentalization will be critical for ascertaining how Epac tightly regulates proteins in various pathways. Are the PRPs specific for NE-associated plasticity, or do they belong to a generic class of proteins that can be recruited by multiple neuromodulatory transmitters? There is evidence hinting at the possible existence of distinct tags during different types of synaptic modifications (e.g., LTP and LTD: Sajikumar and Frey 2004). However, it is still unclear whether different neuromodulators can engage distinct PRPs during heterosynaptic plasticity.

From a biochemical-behavioral perspective, it is known that concentrations of NE (and other monoamines such as dopamine and serotonin) in the rodent hippocampus can be increased by strong electrical stimulation that initiates long-lasting potentiation (Neugebauer et al. 2009). Such sensitivity of the levels of key neuromodulatory transmitters to the pattern of synaptic stimulation may influence processing of behavioral events during associative learning. The main source of NE in the brain, the locus coeruleus, may act cooperatively with other brain structures (e.g., the dopaminergic ventral tegmental area) to control the generation of behavioral tags (analogous to synaptic tags) triggered during learning. These "learning tags" may signal where a memory is stored, whereas protein synthesis, gated by β-AR activation through NE, would allow memories to be consolidated in distinct brain circuits (Moncada 2017). It is known that β-AR activation is needed for triggering PRP synthesis during behavioral tagging; PKA and calcium/calmodulin-dependent protein kinase II (CaMKII) are also needed for setting the learning tags (Moncada et al. 2011). It is unclear whether Epac activation is needed for behavioral tagging and subsequent memory consolidation. Nonetheless, the currently sparse literature on Epac and memory processing supports the notion that Epac has key roles in memory dynamics. Epac activation can modulate associative odor memories in rat pups (Grimes et al. 2015), and Epac signaling is critical for hippocampal memory retrieval (Ouyang et al. 2008; Ostroveanu et al. 2010). Conversely, Epac activation in the amygdala impeded cocaine-induced memory reconsolidation (Wan et al. 2014). Epac expression is dysregulated during Alzheimer's disease (AD; McPhee et al. 2005), but the causal link between Epac and memory dysfunction during AD remains unclear. Insights into the dynamics of Epac in memory-impaired brains could provide a model for noradrenergic signaling during memory processing. Identifying the molecular substrates of Epac and noradrenergic modulation (i.e., NE) will shed light on how cAMP signaling mechanisms, in general, modulate memory consolidation and enable the persistence of associative long-term memories.

Materials and Methods

Animals

Male C57BL/6 mice (Charles River Laboratories, Canada) were used for all experiments. Mice (aged 7–12 wk) were fed ad libitum and housed at the University of Alberta in accordance with Canadian Council on Animal Care (CCAC) guidelines. All reported data were obtained from in vitro hippocampal slices. Proper procedures were taken to minimize animal pain and suffering, as approved by the University of Alberta's Heath Sciences Laboratory Animal Services ethics committee.

Hippocampal slice preparation

Following cervical dislocation and decapitation, the intact mouse brain was removed and placed in ice-cold artificial cerebrospinal fluid (aCSF). Both hippocampi were extracted and sliced transversely (at 400 µm thickness) using a tissue chopper. Slices were transferred to an interface chamber (Fine Science Tools, Canada), where they recovered for ∼90 min at 30°C and aerated with carbogen (95% O_2/5% CO_2). Slices were continuously perfused with aCSF at a rate of 1 mL per min. Our aCSF contained (in mM): 124 NaCl, 4.4 KCl, 1.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 10 glucose.

Electrophysiology

fEPSPs were recorded from the CA1 area by placing stimulating and recording electrodes in the stratum radiatum. Recording glass microelectrodes were filled with aCSF (resistance of 2–3 MΩ), and bipolar nickel–chromium electrodes were used for stimulating the Schaffer collateral-commissural pathway. Two independent synaptic paths (S1 [homosynaptic pathway] and S2 [heterosynaptic pathway]) were stimulated; both converge onto the same population of CA1 pyramidal neurons. Both pathways were confirmed to be separate because they did not exhibit inter-pathway paired-pulse facilitation (Connor et al. 2011). fEPSPs were evoked at a stimulus intensity of 40% of their maximum amplitude, measured in mV. fEPSPs were elicited at a test stimulation frequency of once per minute (0.08 msec pulse duration) by stimulating both pathways 200 msec apart.

Drugs

Following 20 min of baseline recordings at test stimulation frequency, different protocols were administered involving drug application and/or high-frequency stimulation (one train of 100 Hz at 1 sec train duration) to one pathway or both. This 100 Hz protocol was often paired with L-(−)- NE (+)-bitartrate salt monohydrate (NE) (10 µM; Sigma) applied at S1. Induction of homosynaptic and heterosynaptic LTP by pairing one train of 100 Hz with ISO has been previously described (Gelinas and Nguyen 2005; Connor et al. 2011). In the present experiments, NE was applied 10 min before one train (1 sec) of 100 Hz at S1, followed by an additional 5 min post-100 Hz. A PKA inhibitor, KT-5720 (KT) (1 µM; Sigma), was dissolved in a stock concentration of 1 mM in dimethyl sulfoxide (DMSO). PKA inhibitor fragment (PKI 1 µM; 14–22 amide, myristoylated membrane-permeant from Calbiochem) was prepared as a 1 mM stock solution in distilled water. KT was added 20 min prior to application of NE at S1; PKI was perfused for 20 min before NE application. The Epac inhibitors, ESI-09 (ESI; α-[2-(3-chlorophenyl)hydrazinylidene]-5-(1,1-dimethylethyl)-b-oxo-3-isoxazolepropanenitrile) (10 µM; Sigma) and BFA (10 µM; Sigma), were dissolved in DMSO at 20 mM and 10 mM, respectively. Each inhibitor remained present until 5 or 10 min after NE washout (35–45 min total drug application). Drugs which overlapped with one train (1 sec duration) of 100 Hz at S2 were applied starting at 20

min before 100 Hz (with the exception of KT, which was perfused for 10 min prior to 100 Hz) and continued for an additional 5 min. Experiments were conducted under dimmed light conditions to minimize photolysis of light-sensitive drugs such as NE and some inhibitors. Drug experiments were interspersed with drug-free controls.

Statistical analysis

Data were analyzed offline using pCLAMP 10 software (Axon Instrument Inc.). Initial fEPSP slopes were measured as an index of synaptic strength (Johnston and Wu 1995). Slopes were averaged from 20 min of steady baseline recording (prior to 100 Hz) to obtain a "baseline" mean value for each experiment. All subsequent slopes were expressed as percentages of these baseline mean slopes. Mean fEPSP slopes, measured 90 min after 100 Hz in S2, were used for inter-group comparisons of synaptic strength. Student's *t*-test was used for statistical comparisons of mean fEPSP slopes between two groups. One-way ANOVA and Tukey–Kramer post-hoc tests were used for comparing the significance between three or more groups (significance level of $P < 0.05$). All values were standardized to the average baseline slope and reported as mean ± SEM.

Acknowledgments

We thank Ms. J. Hoffman for her assistance with some of the slice experiments. This research was supported by a Discovery Grant (to P.V.N.) from the Natural Sciences and Engineering Research Council of Canada. N.J.B. received graduate support from this grant.

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Received October 31, 2018; accepted in revised form November 30, 2018.