

# Hippocampal Long-Term Potentiation That is Elicited by Perforant Path Stimulation or That Occurs in Conjunction with Spatial Learning is Tightly Controlled by Beta-Adrenoreceptors and the Locus Coeruleus

Niels Hansen and Denise Manahan-Vaughan\*

**ABSTRACT:** The noradrenergic system, driven by locus coeruleus (LC) activation, plays a key role in the regulating and directing of changes in hippocampal synaptic efficacy. The LC releases noradrenaline in response to novel experience and LC activation leads to an enhancement of hippocampus-based learning, and facilitates synaptic plasticity in the form of long-term depression (LTD) and long-term potentiation (LTP) that occur in association with spatial learning. The predominant receptor for mediating these effects is the  $\beta$ -adrenoreceptor. Interestingly, the dependency of synaptic plasticity on this receptor is different in the hippocampal subfields whereby in the CA1 *in vivo*, LTP, but not LTD requires  $\beta$ -adrenoreceptor activation, whereas in the mossy fiber synapse LTP and LTD do not depend on this receptor. By contrast, synaptic plasticity that is facilitated by spatial learning is highly dependent on  $\beta$ -adrenoreceptor activation in both hippocampal subfields. Here, we explored whether LTP induced by perforant-path (pp) stimulation *in vivo* or that is facilitated by spatial learning depends on  $\beta$ -adrenoreceptors. We found that under both LTP conditions, antagonising the receptors disabled the persistence of LTP.  $\beta$ -adrenoreceptor-antagonism also prevented spatial learning. Strikingly, activation of the LC before high-frequency stimulation (HFS) of the pp prevented short-term potentiation but not LTP, and LC stimulation after pp-HFS-induced depotentiation of LTP. This depotentiation was prevented by  $\beta$ -adrenoreceptor-antagonism. These data suggest that  $\beta$ -adrenoreceptor-activation, resulting from noradrenaline release from the LC during enhanced arousal and learning, comprises a mechanism whereby the duration and degree of LTP is regulated and fine tuned. This may serve to optimize the creation of a spatial memory engram by means of LTP and LTD. This process can be expected to support the special role of the dentate gyrus as a crucial subregional locus for detecting and processing novelty within the hippocampus. © 2015 The Authors Hippocampus Published by Wiley Periodicals, Inc.

**KEY WORDS:** rat; hippocampus; long-term potentiation; learning-facilitated long-term potentiation; beta-adrenergic receptors

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## INTRODUCTION

Noradrenaline modulates hippocampal synaptic plasticity via the activation of  $\beta$ -adrenoreceptors (Gelinas and Nguyen, 2005; Kemp and Manahan-Vaughan, 2008a,2008b; Hagen and Manahan-Vaughan, 2012; Goh et al., 2013). The main source of noradrenaline in the brain is the locus coeruleus (LC), a structure located in the dorsal pons. Novel experience drives activation of the LC (Sara et al., 1994; Vankov et al., 1995) and activation of  $\beta$ -adrenoreceptors is a key factor in the detection of a novel stimulus by brain structures such as the hippocampus.

Long-term potentiation (LTP) and long-term depression (LTD) are cellular memory storage mechanisms (Bliss and Collingridge, 1993; Kemp and Manahan-Vaughan, 2007; Nabavi et al., 2014). The activation of  $\beta$ -adrenoreceptors modulates LTP in the hippocampal CA1, CA3, and dentate gyrus subregions *in vivo* (Straube et al., 2003; Kemp and Manahan-Vaughan, 2008a,2008b; Hagen and Manahan-Vaughan, 2012), as well as *in vitro* (Huang and Kandel, 1996; Bramham et al., 1997; Swanson-Park et al., 1999). Strikingly, activation of the LC induces input-specific synaptic plasticity in the form of LTD in both the CA1 and dentate gyrus (Lemon et al., 2009; Hansen and Manahan-Vaughan, *in press*). It also enhances evoked responses the dentate gyrus (Harley, 1991; Walling and Harley, 2004; Walling et al., 2004; Lashgari et al., 2008; Rajkumar et al., 2013), thus suggesting that it has a direct influence on information processing and storage in this structure. On a functional level, memory formation and consolidation (Gibbs et al., 2010), objection recognition memory (Mello-Carpes and Izquierdo, 2013) and emotional memory (Tully and Bolshakov, 2010) are also modulated via the noradrenergic LC system. This suggests an important contribution of the noradrenergic system to hippocampus-based learning and memory, as well as its underlying processes.

$\beta$ -adrenoreceptors are expressed in all hippocampal subregions to a differing extent (Booze et al., 1993; Milner et al., 2000; Guo and Li, 2007; Cox et al.,

2008) suggesting that noradrenergic control of the hippocampal subfields may be graded relative to their role in processing novel stimuli and information encoding. The dentate gyrus is particularly interesting in this regard. As a recipient of a greater density of LC noradrenergic fiber projections to the dentate gyrus than to other hippocampal subregions (Loy et al., 1980; Fallon and Loughlin, 1987), it may be subject to greater control by the LC and noradrenaline. Subregional differences with regard to  $\beta$ -adrenoreceptor-mediated regulation of synaptic plasticity have been reported. Whereas  $\beta$ -adrenoreceptors are required for LTP that is induced by high-frequency stimulation (HFS) of Schaffer-collateral CA1 synapses *in vivo*, they are not required for HFS-induced LTP in mossy fiber-CA3 synapses (Kemp and Manahan-Vaughan, 2008a,2008b; Hagen and Manahan-Vaughan, 2012). Noradrenaline is released in the dentate gyrus after HFS of the medial perforant path, and is associated with hippocampal LTP in the DG *in vivo* (Bronzino et al., 2001). The induction and maintenance of HFS-induced LTP is regulated by  $\beta$ -adrenoreceptors in the dentate gyrus *in vitro* (Bramham et al., 1997; Swanson-Park et al., 1999) and *in vivo* (Munro et al., 2001; Straube et al., 2003). In the dentate gyrus, very persistent forms of LTP also require  $\beta$ -adrenoreceptors, although this dependency can be overcome by the induction of very robust LTP (Straube and Frey, 2003).

Another form of very persistent (>24 h) LTP has been described that is triggered by the combination of weak HFS (that induces transient short-term potentiation, STP) with the learning of novel scenes or changes in global space (Kemp and Manahan-Vaughan, 2004). In mossy fiber-CA3 or Schaffer collateral-CA1 synapses, this type of LTP, that is referred to as learning-facilitated LTP (Kemp and Manahan-Vaughan, 2007) requires activation of  $\beta$ -adrenoreceptors in order for successful expression to occur (Kemp and Manahan-Vaughan, 2008a,2008b; Hagen and Manahan-Vaughan, 2012). It is not known to what extent  $\beta$ -adrenoreceptors are required for this kind of learning-facilitated LTP in the dentate gyrus. However, novel exploration comprising movement from a familiar chamber to an identical novel chamber, that strengthens STP into LTP, has been reported to rely on activation of  $\beta$ -adrenoreceptors (Straube et al., 2003).

In the present study we explored whether  $\beta$ -adrenoreceptors regulate LTP that is induced by HFS, or LTP that is facilitated by spatial learning. We also explored whether LTP is modulated by stimulation of the LC. Given the importance of the dentate gyrus for pattern separation (Rolls et al., 2010; Kesner et al., 2013b) and observations that noradrenaline release following arousal supports pattern separation in the dentate gyrus (Segal et al., 2012), we postulated that  $\beta$ -adrenoreceptors will be critically involved in the enablement of dentate gyrus LTP that persists for long periods, as well as LTP that is facilitated by hippocampus-dependent learning. Our data show that both forms of LTP require  $\beta$ -adrenoreceptor activation, in line with an important regulatory role of arousal and noradrenaline release for information processing in the dentate gyrus. In addition, we show that the duration of synaptic potentiation is tightly controlled by the LC: a process that may support the

integration of spatial experience into a synaptic ensemble/engram by means of LTP and LTD.

## MATERIALS AND METHODS

All experiments were conducted in agreement with the European Communities Council Directive of September 22nd, 2010 (2010/63/EU) for the care of laboratory animals with prior approval from the local ethics committee (Bezirksamt Arnsberg). All measures were taken to minimize animal suffering and the number of animals used.

### Surgery

We implanted hippocampal electrodes and a guide cannula in anesthetized male Wistar rats (Charles River, Sulzfeld, Germany, 7–8 weeks old) as described previously (Manahan-Vaughan, 1997). Briefly, following anesthetization with sodium pentobarbital (Synopharm, Germany) (“nembutal,” 52 mg/kg, intraperitoneally), the animals underwent implantation of a monopolar recording and bipolar stimulating electrode [made of 0.1 mm diameter Teflon-coated stainless-steel wire (Biomedical Instruments, Zöllnitz, Germany)] attached outside the skull in order to secure the wire above it. A drill hole (1 mm diameter) was made for the recording electrode; a second drill hole (1 mm diameter) for the stimulation electrodes. On the contralateral side, two holes were drilled (1.2 mm in diameter) into which anchor screws were set. Moreover, the anchor screws also were conducted to reference or ground electrodes. The recording electrode was placed in the dentate gyrus granule cell layer (3.1 mm posterior to bregma and 1.9 mm lateral to the midline) and the stimulation electrode was placed in the medial perforant path (6.9 mm posterior to bregma and 4.1 mm lateral to the midline) as described previously (Hansen and Manahan-Vaughan, *in press*). This enabled dual recordings of the field excitatory postsynaptic potential (fEPSP) and the somatic population spike (PS), so that we could verify that LTP was accompanied by epsp-spike (E-S) potentiation (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973).

For intracerebroventricular (i.c.v.) injections, a cannula was implanted into the lateral cerebral ventricle (0.5 mm posterior to bregma, 1.6 mm lateral to the midline; size: 5.6 mm length, 0.8 mm diameter, 4.5 mm depth). The electrodes' correct location was verified during the implantation procedure by the electrophysiological characteristics of the field potentials evoked. A third hole was drilled ipsilateral to the hippocampal electrodes, to insert a bipolar stimulation electrode in the LC (6.9 mm ventral to dura matter, entering at a 15° angle to the skull's plane) with the following coordinates: 3 mm posterior and 1.2 mm lateral to lambda, as described previously (Lemon et al., 2009).

The following criteria were used to distinguish potentials elicited by medial perforant path (PP) stimulation from lateral path stimulation (Abraham and McNaughton, 1984): an fEPSP

peak latency of  $\sim 3$  ms and half-width of  $\sim 5$  ms and the presence of PS within the first positive deflection of the fEPSP. The entire assembly was sealed and secured to the skull with dental acrylic (Paladur, Heraeus Kulzer GmbH, Hanau, Germany). Ten days after surgery, recordings were obtained in the DG granule cell layer by stimulating the medial PP. During the experiments, the animals moved freely within the recording chamber ( $40 \times 40 \times 40$  cm), as the implanted electrodes were connected via a flexible cable and swivel connector to the stimulation unit and amplifier. Aside from insertion of the connector cable at the start of the experiment, disturbing the animals was kept to an absolute minimum. During the experiments, each animal's electroencephalogram (EEG) was monitored continuously.

### Measurement of Evoked Potentials

To measure synaptic activity in the DG, we analyzed both the PS amplitude and fEPSP slope. To obtain these measurements, an evoked response was generated by stimulating at low frequency (0.025 Hz) with single biphasic square wave pulses of 0.2 ms duration per half wave, generated by a constant current isolation unit. For each time-point measured during the experiments, we averaged five recordings of evoked responses. The first six time-points recorded at 5 min intervals served as our "baseline" reference values, and data points obtained thereafter were calculated as a percentage of the mean of those six points. The fEPSP was measured by the maximum slope through the five steepest points obtained on the first positive deflection of the potential and maximum of the second positive deflection. The PS amplitude was measured from the peak of the first positive deflection (PS onset) to the peak of the potential's first negative deflection (PS peak). PS amplitude indicates the summated action potentials of granule cells in the DG's somatic layer, whereas the fEPSP slope depicts alterations in the DG's dendritic excitability. By determining the input/output curve (evaluating nine different stimulation intensities from 100 to 900  $\mu\text{A}$  in 100  $\mu\text{A}$  steps), we identified the maximum PS amplitude, and all potentials employed as baseline criteria during the experiments were evoked at a stimulus intensity that produced 40% of this maximum. Five such evoked responses were recorded every 40 s, and averaged and repeated every 5 min to ensure a representative average recording for each 5-min interval. After 90 min of recording, the interval between samples of evoked potentials was extended to 15 min.

LTP was induced by afferent high-frequency stimulation of medial perforant path (HFS; 10 bursts of 15 pulses at 200 Hz with 10 s interburst intervals). To evoke a short-term potentiation (STP,  $< 2$  h), a subthreshold HFS (sub-HFS) consisting of three bursts of 15 pulses at 200 Hz with 10 s interburst intervals was given. To induce short-term potentiation that persists for less than 1 h, we administered weak HFS (wHFS) to the medial perforant path comprising 100 Hz ( $10 \times 10$  bursts, 10 ms stimulus interval, stimulus duration 0.1 ms).

### Locus Coeruleus Stimulation

LC stimulation comprised two trains of 100 pulses at 100 Hz with each train lasting one second with a 20-s intertrain

interval. This was previously shown to modulate LTD in the dentate gyrus (Hansen and Manahan-Vaughan, in press). The stimulus strength comprised  $62 \pm 21 \mu\text{A}$  given as single biphasic square-wave pulses, of 0.1 ms duration per half-wave. We determined the stimulus strength individually for each animal during a preliminary experiment. Stimuli in the range of 20 to 100  $\mu\text{A}$  were applied. The stimulation intensity for LC activation in each animal was set as the intensity that was subthreshold for the triggering of a stress response (Lemon et al., 2009). This test was performed in an open field arena (width: 100 cm, length: 100 cm, height: 50 cm) 1 week before experimental recordings were commenced (Lemon et al., 2009; Hansen and Manahan-Vaughan, in press). In LC experiments animal received test-pulse stimulation to evoke basal synaptic transmission, and LC stimulation was applied about 60 min after recordings had been commenced. In one cohort, LC stimulation preceded wHFS in another it succeeded HFS. Where vehicle, or propranolol, was applied, this occurred 30 min before LC stimulation or HFS. Animals served as their own controls: the separate experiments were spaced by about 7 days.

### Novel Spatial Exploration

Seven days after the animals received sub-HFS, a holeboard ( $39.8 \times 39.8$  cm, washable gray plastic) that contained a hole in each quadrant (Kemp and Manahan-Vaughan, 2004), was placed onto the floor of the recording chamber. This was done just before applying sub-HFS. The holeboard was removed 10 min after sub-HFS. After 7 days, the animals were re-exposed to the same holeboard and sub-HFS was given a new.

### Statistics

To analyze differences between groups, we conducted a two-way mixed analysis of variance (ANOVA) with the between-group factor being pharmacological treatment, or holeboard exploration, and "time-after-stimulation" being the within-group factor. We report only the significant interaction effects between the treatment and time-after-stimulation factors. All data periods are expressed as a mean percentage  $\pm$  SEM of the average baseline value. The level of significance was set at  $P < 0.05$ .

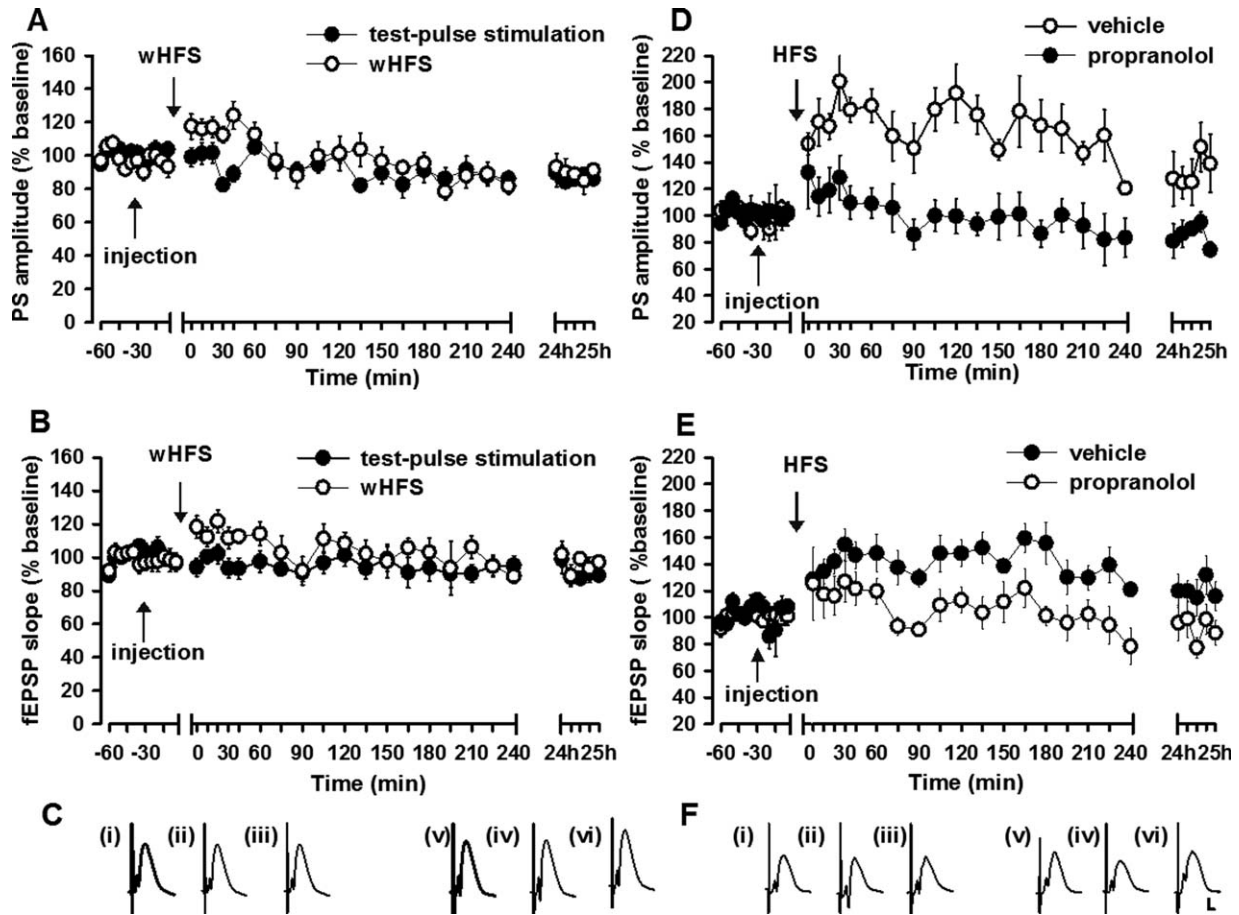
### Histology

At the end of the experiments, brains were removed to histologically verify the location of the electrodes and cannula as described previously (Hansen and Manahan-Vaughan, in press). Photomicrographs were taken with a digital video camera system (Visitron Systems, Puchheim, Germany) on a Leica DM LB Microscope (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Brains in which the electrodes had been incorrectly implanted were excluded from the study.

### Compounds and Drug Treatment

The  $\beta$ -adrenoreceptor antagonist propranolol (2  $\mu\text{g}$ ) and  $\beta$ -adrenoreceptor agonist isoproterenol (20  $\mu\text{g}$ ) (Tocris





**FIGURE 1.** Long-term potentiation that is induced in the dentate gyrus by patterned stimulation of the perforant path requires  $\beta$ -adrenoreceptor activation. (A, B) Weak high frequency stimulation (wHFS; 100 Hz) causes short-term potentiation in the dentate gyrus (DG) that lasts for less than 1 h. (D, E) High frequency stimulation (HFS, 10 trains at 200 Hz) induces long-term potentiation (LTP) in the DG that lasts for over 24 h. Prior treatment with the  $\beta$ -adrenoreceptor antagonist propranolol (2  $\mu$ g) significantly attenuates this LTP. The mean population spike (PS) amplitude (A, D) and mean fEPSP slope (B, E) are shown along with

the corresponding standard error of the mean (SEM). (C) Analog traces represent perforant path-DG field potentials (i) 5 min before, (ii) 5 min after, and (iii) 24 h after vehicle and test-pulse stimulation, or (iv) 5 min before, (v) 5 min after and (vi) 24 h after vehicle and weak HFS stimulation. (F) Analog traces represent perforant path-DG field potentials (i) 5 min before, (ii) 5 min after, and (iii) 24 h after vehicle in the presence of HFS stimulation, or (iv) 5 min before, (v) 5 min after and (vi) 24 h after propranolol in the presence of HFS stimulation. Vertical bar, 3 mV; horizontal bar, 2.5 ms.

Bioscience, UK), or vehicle (0.9% NaCl) were injected into the ipsilateral intracerebral ventricle by means of the implanted cannula as a 5  $\mu$ L volume over 5 min, concluding 25 min before HFS or sub-HFS were given. We used these concentrations because do not alter basal synaptic transmission in the hippocampus in vivo (Kemp and Manahan-Vaughan, 2008a,2008b).

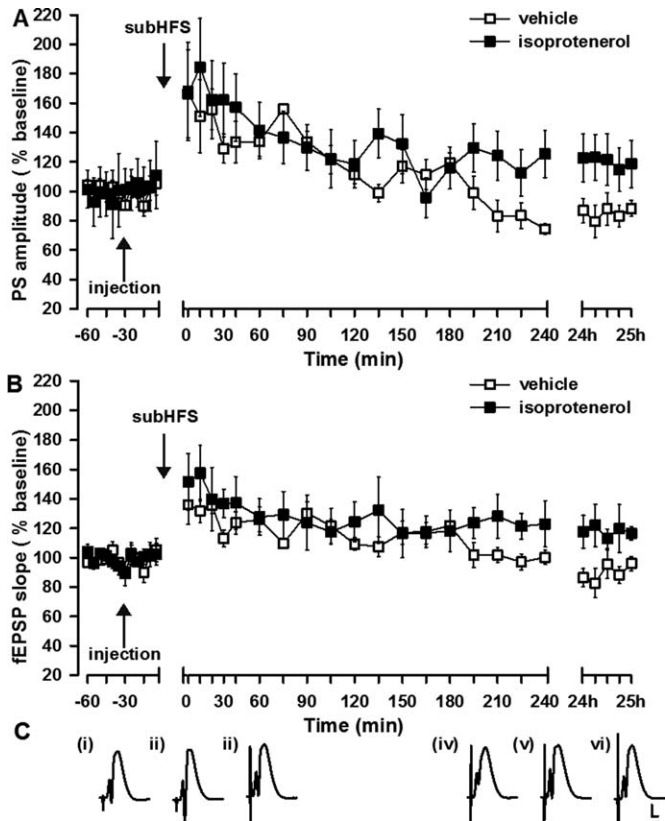
## RESULTS

### Long-Term Potentiation in the Dentate Gyrus In Vivo Requires Activation of $\beta$ -Adrenoreceptors

Weak high frequency stimulation (wHFS) at 100 Hz elicited a small and transient short-term potentiation (STP) in the den-

tate gyrus of freely moving rats that lasted 45 min compared with animals that received test-pulse stimulation (Figs. 1A–C) (PS: ANOVA,  $F_{(1,18)} = 22.3$ ,  $P < 0.001$ ; fEPSP: ANOVA,  $F_{(1,18)} = 1.9$ ,  $P < 0.01$ ;  $n = 10$  in both groups). Raising the stimulation frequency to 200 Hz HFS resulted in LTP that lasted for at least 24 h compared with control animals that received test-pulse stimulation (Figs. 1D–F) (PS: ANOVA,  $F_{(1,14)} = 59.4$ ,  $P < 0.0001$ ; fEPSP: ANOVA,  $F_{(1,14)} = 34.9$ ,  $P < 0.0001$ ;  $n = 8$  in both groups).

When the  $\beta$ -adrenoreceptor antagonist, propranolol (2  $\mu$ g), was applied before 200 Hz HFS (10 bursts) a significant impairment of LTP became apparent. LTP deficits became significant 30 min after HFS (Figs. 1D–F) (PS: ANOVA:  $F_{(1,12)} = 22.4$ ,  $P < 0.005$ ; fEPSP, ANOVA:  $F_{(1,12)} = 9.9$ ,  $P < 0.05$ ;  $n = 8$  vehicle vs.  $n = 6$  propranolol-treated animals). Twenty-four hours after HFS, evoked potentials were at pre-HFS levels in propranolol-treated animals and were equivalent



**FIGURE 2.** Long-term potentiation is facilitated by activating  $\beta$ -adrenoreceptors in the dentate gyrus. (A, B) Subthreshold-high frequency stimulation (sub-HFS, three trains at 200 Hz) elicits short-term potentiation (STP) that lasts about 3 h in vehicle-treated animals. Prior treatment with the  $\beta$ -adrenoreceptor agonist isoproterenol (20  $\mu$ g) facilitates STP into LTP that endures for at least 25 h (A, B). (C) Analog traces represent perforant path-dentate gyrus (DG) field potentials (i) 5 min before, (ii) 5 min after, and (iii) 24 h after vehicle in the presence of sHFS, or (iv) 5 min before, (v) 5 min after, and (vi) and 24 h after isoproterenol in the presence of sHFS. Calibration: Vertical bar, 3 mV; horizontal bar, 2.5 ms.

to responses evoked in animals that received test-pulse stimulation only (24 h: PS, ANOVA,  $F_{(1,12)} = 0.002$ ,  $P = 0.96$ ; fEPSP, ANOVA  $F_{(1,12)} = 0.02$ ;  $P = 0.89$ ;  $n = 8$  in vehicle and  $n = 6$  in propranolol-treated animals, Figs. 1D–F).

### Activation of $\beta$ -Adrenoreceptors Converts Short-Term Potentiation Into LTP in the Dentate Gyrus In Vivo

Having observed that antagonism of  $\beta$ -adrenoreceptors prevents LTP in the dentate gyrus in vivo, we explored whether activation of  $\beta$ -adrenoreceptor can strengthen or prolong STP. Here, we used three, instead of 10, bursts of 200 Hz HFS that results in STP that lasts for about 2 h (“subthreshold” high-frequency stimulation; sub-HFS) in control animals (Figs. 2A,B) (PS: ANOVA,  $F_{(1,12)} = 14.1$ ,  $P < 0.005$ ; fEPSP, ANOVA  $F_{(1,12)} = 26.4$ ;  $P < 0.0005$  compared with test-pulse stimulated controls;  $n = 7$  in both groups, When the  $\beta$ -

adrenoreceptor agonist, isoproterenol (20  $\mu$ g), was administered before subHFS, STP was strengthened into LTP that endured for at least 25 h (210 min–25 h, PS amplitude: ANOVA  $F_{(1,12)} = 9.9$ ,  $P < 0.05$ ; fEPSP: ANOVA,  $F_{(1,12)} = 6.7$ ;  $P < 0.05$ ;  $n = 7$  in both groups, Figs. 2A,B).

### Locus Coeruleus Stimulation Before High-Frequency Stimulation Prevents Short-Term but not Long-Term Potentiation

The finding that antagonism of  $\beta$ -adrenoreceptors prevents LTP whereas the activation of  $\beta$ -adrenoreceptor enhances STP into LTP, was an intriguing observation, given the finding that LC stimulation results in input-specific  $\beta$ -adrenoreceptor-dependent LTD in the dentate gyrus in vivo (Hansen and Manahan-Vaughan, in press). Thus we explored how LC stimulation affects STP (<45 min) and LTP (>25 h) in the dentate gyrus of freely behaving rats.

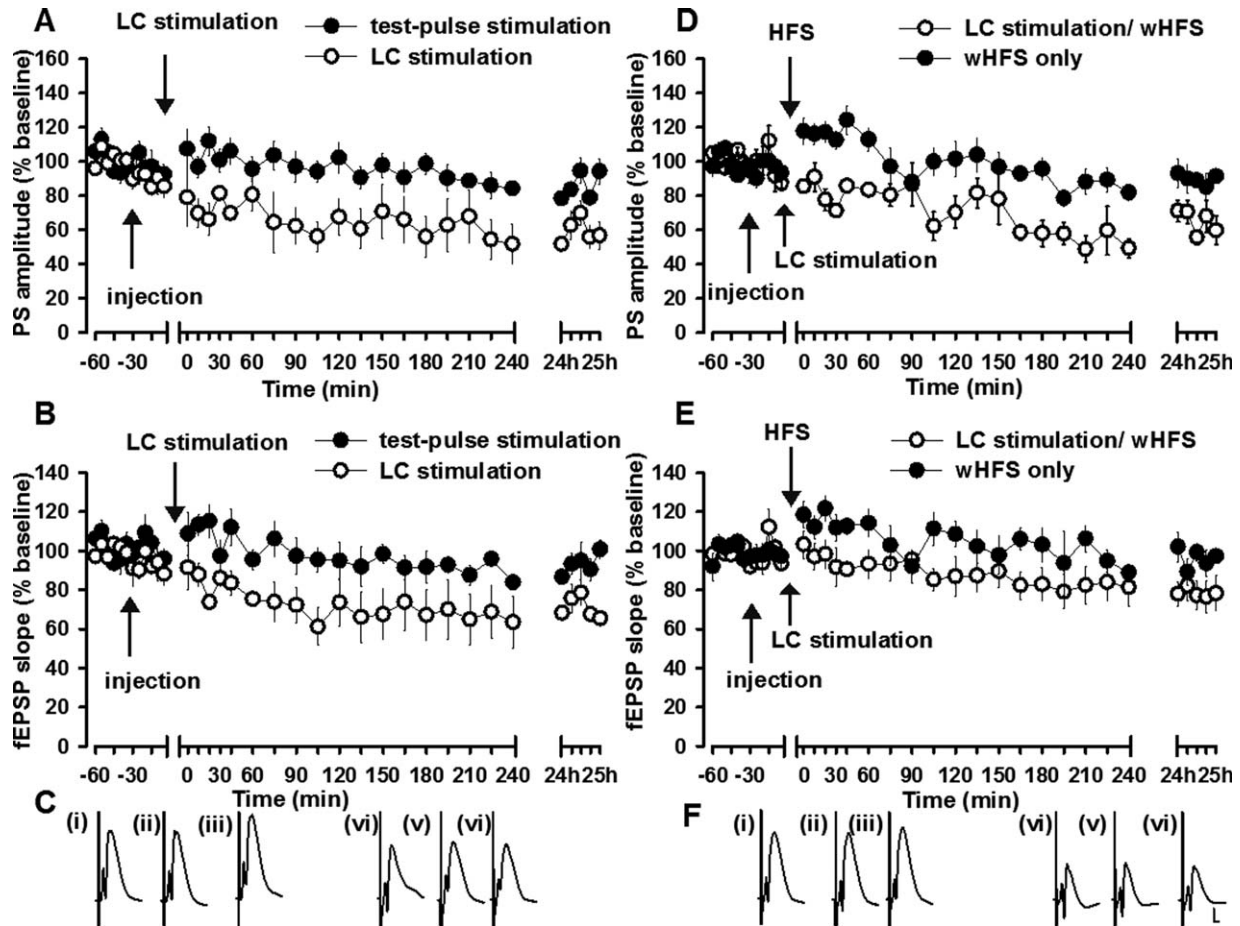
In animals that received test-pulse stimulation to the perforant path (pp), LC stimulation resulted in a persistent synaptic depression in the DG that lasted for over 24 h (Figs. 3A–C; LC stimulation/vehicle compared with test-pulse stimulated animals; PS:  $F_{(1,10)} = 19.8$ ,  $P < 0.05$ ; fEPSP:  $F_{(1,10)} = 11.2$ ,  $P < 0.01$ ; LC stimulation/vehicle  $n = 6$ ; test-pulse  $n = 6$ ). When LC stimulation was applied before wHFS, short-term potentiation was significantly impaired (Figs. 3D–F; LC/wHFS compared with wHFS-stimulated controls, 45 min; PS:  $F_{(1,14)} = 71.3$ ,  $P < 0.001$ ; fEPSP:  $F_{(1,14)} = 6.6$ ,  $P < 0.05$ ; wHFS  $n = 10$ , LC/wHFS  $n = 6$ ). In fact, LC stimulated animals expressed LTD despite having received wHFS to pp synapses (Figs. 3D–F) (165 min to 24 h after HFS, PS:  $F_{(1,14)} = 23.9$ ,  $P < 0.001$ ; 24 to 25 h after HFS, fEPSP:  $F_{(1,14)} = 4.9$ ,  $P < 0.05$ ; HFS  $n = 10$ , LC stimulation before HFS  $n = 6$ ).

Persistent LTP (>25 h) induced by HFS (200 Hz) was highly significant compared with rats that received pp test-pulse stimulation only (Figs. 4A,B,D) (PS:  $F_{(1,10)} = 22.5$ ,  $P < 0.001$ ; fEPSP:  $F_{(1,10)} = 26.6$ ,  $P < 0.001$ ; both groups  $n = 6$ ). When the LC was stimulated before HFS, this caused no alteration in the profile of LTP (PS:  $F_{(1,10)} = 0.01$ ,  $P = 0.9$ ; fEPSP:  $F_{(1,10)} = 0.1$ ,  $P = 0.73$ ; LC stimulation before HFS  $n = 6$ ; HFS  $n = 6$ ; Figs. 4A,B,D).

These data suggest that LC activation may support the rapid decay of weakly potentiated synapses, whilst leaving robustly potentiated synapses intact. This may function to optimise signal-to noise-ratios during hippocampal information encoding.

### LC Stimulation After High-Frequency Stimulation Depotentiates Long-Term Potentiation and in a $\beta$ -Adrenoreceptor-Dependent Manner

The timing of LC activity may be critical to the stability of hippocampal synaptic plasticity and the direction of change of synaptic strength elicited during learning events (Lemon et al, 2009). Here, we explored if LC activation after HFS influences the stability of LTP.



**FIGURE 3.** Locus coeruleus stimulation before weak high-frequency stimulation prevents short-term potentiation of perforant path-dentate gyrus synapses. (A, B) Locus coeruleus (LC) stimulation leads to long-term depression (LTD) in the dentate gyrus (DG) compared with test-pulse stimulated controls. When LC stimulation was applied before weak high-frequency stimulation (wHFS) (100 Hz), short-term potentiation (STP) was significantly impaired (D, E). By 24 h after LC stimulation, LTD was apparent compared with animal that received wHFS only. (C) Analog traces represent perforant path-dentate gyrus (DG) field

potentials (i) 5 min before, (ii) 5 min after, and (iii) 24 h after vehicle in the presence of test pulse stimulation, or (iv) 5 min before, (v) 5 min after, and (vi) 24 h after vehicle in the presence of LC stimulation. (F) Analog traces represent perforant path dentate gyrus (DG) field potentials (i) 5 min before, (ii) 5 min after, and (iii) 24 h after vehicle in the presence of HFS, or (iv) 5 min before, (v) 5 min after, and (vi) 24 h after vehicle in the presence of prior LC stimulation to HFS. Vertical bar, 3 mV; horizontal bar, 2.5 ms.

In another animal cohort, HFS protocol (200Hz) induced robust LTP compared with test-pulse stimulated animals (PS:  $F_{(1,20)} = 31.01$ ,  $P < 0.0001$ ; fEPSP:  $F_{(1,20)} = 50.9$ ,  $P < 0.0001$ ; both groups  $n = 11$ ). However, when the LC was stimulated after HFS, depotentiation of LTP occurred (PS:  $F_{(1,20)} = 15.3$ ,  $P < 0.001$ ; fEPSP:  $F_{(1,20)} = 27.6$ ,  $P < 0.0001$  compared with pp-HFS only; both groups  $n = 11$ ; Figs. 4E–G).

Effects were  $\beta$ -adrenoreceptor-dependent: prior application of the antagonist propranolol prevented LC-mediated depotentiation compared with vehicle-treated controls (PS:  $F_{(1,14)} = 9.2$ ,  $P < 0.01$ ; fEPSP:  $F_{(1,24)} = 12.3$ ,  $P < 0.005$ ; vehicle  $n = 11$ ; LC propranolol  $n = 5$ ; Figs. 4E–G). The depotentiation was completely reversed, so that the LTP did not differ between those animals that received vehicle/HFS and

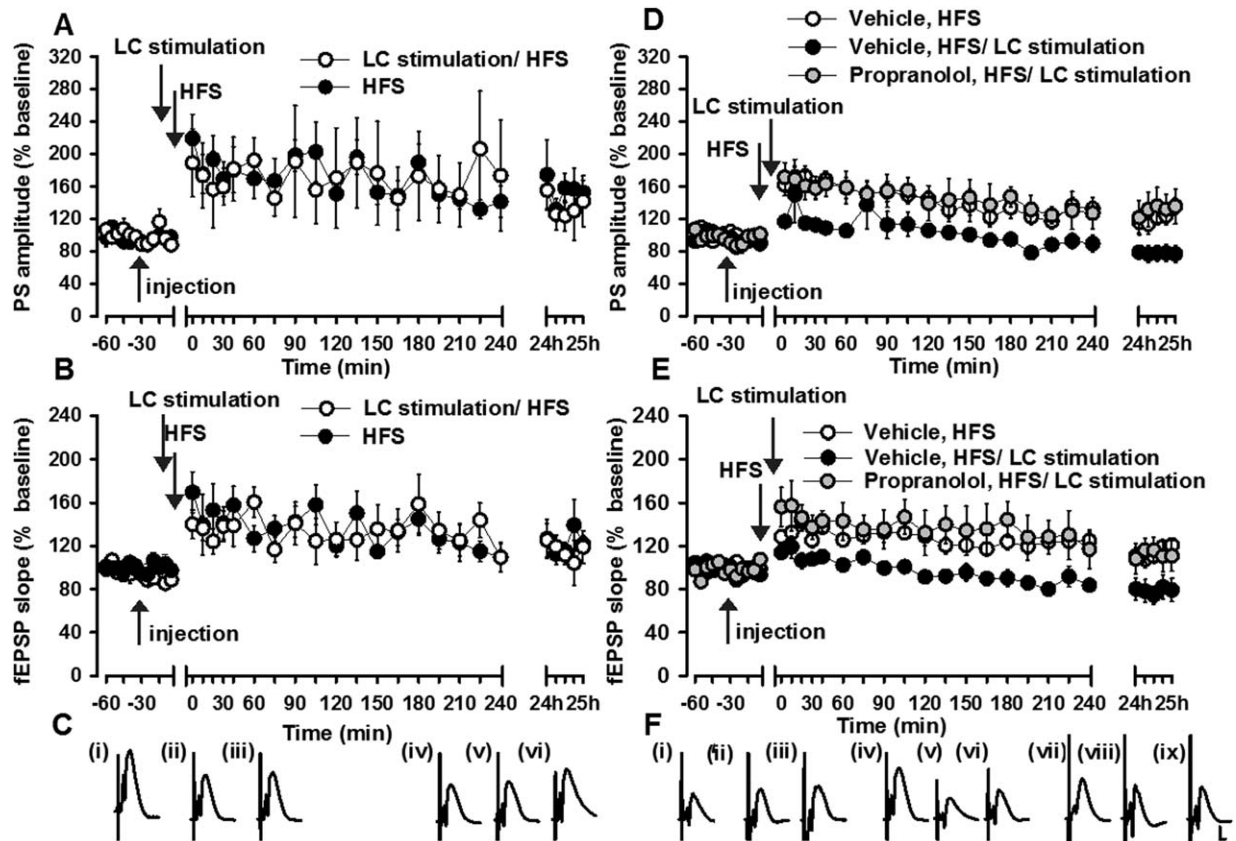
those treated with propranolol before HFS and LC stimulation (PS:  $F_{(1,14)} = 0.06$ ,  $P = 0.8$ ; fEPSP:  $F_{(1,14)} = 0.59$ ,  $P = 0.45$ ; HFS with vehicle  $n = 11$ ; LC stimulation after HFS with propranolol  $n = 5$ ; Figs. 4E–G).

### Learning About a Novel Environment Facilitates Long-Term Potentiation at Perforant Path-Dentate Gyrus Synapses. Effects are Prevented by Antagonism of $\beta$ -Adrenoreceptors

Having found that beta-adrenoreceptors exert such a potent influence on DG LTP, we explored whether these receptors influence LTP that is facilitated in the DG by learning.

Previous studies have shown that the exploration of a novel empty holeboard during sub-HFS application facilitates





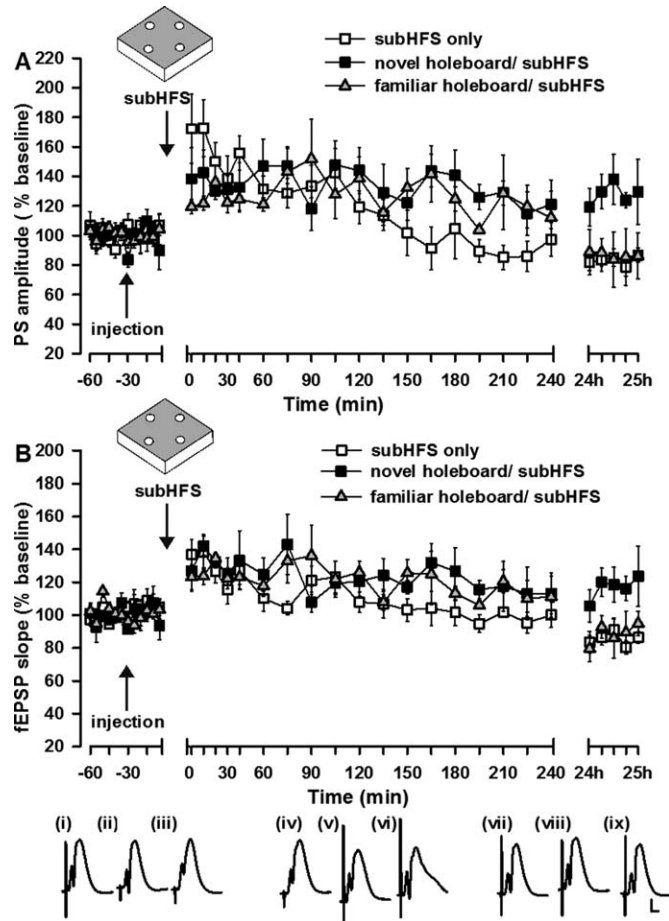
**FIGURE 4.** Long-term potentiation of perforant path-dentate gyrus synapses is depotentiated by subsequent, but not antecedent, locus coeruleus stimulation. (A, B) Strong high frequency stimulation (HFS: 10 trains at 200 Hz) induces long-term potentiation (LTP) in dentate gyrus that lasts for over 25 h. Stimulation of the LC before HFS does not alter the profile of LTP. (D, E) LC stimulation after HFS results in significant depotentiation. Treatment with the  $\beta$ -adrenoreceptor antagonist propranolol (2  $\mu$ g) before HFS/LC stimulation significantly prevents depotentiation. (C) Analog traces represent perforant path-dentate gyrus (DG) field poten-

tials (i) 5 min before, (ii) 5 min after, and (iii) 24 h after vehicle in the presence of HFS or (iv) 5 min before, (v) 5 min after, and (vi) and 24 h after vehicle in the presence of LC stimulation before HFS. (F) Analog traces represent perforant path dentate gyrus (DG) field potentials (i) 5 min before, (ii) 5 min after, and (iii) 24 h after vehicle in the presence of HFS, (iv) 5 min before, (v) 5 min after, and (vi) and 24 h after vehicle in the presence of LC stimulation after HFS and (vii) 5 min before, (viii) 5 min after, and (ix) and 24 h after propranolol in the presence of LC stimulation after HFS. Vertical bar, 3 mV; horizontal bar, 2.5 ms.

STP into LTP (Kemp and Manahan-Vaughan, 2004, 2008b; Uzakov et al., 2005; Hagena and Manahan-Vaughan, 2011; Kenney and Manahan-Vaughan, 2013). This property has been observed in mossy fiber-commissural-associational synapses (Hagena and Manahan-Vaughan, 2011), perforant path-dentate gyrus synapses and Schaffer collateral-CA1 synapses (Kemp and Manahan-Vaughan, 2004, 2008b). We observed the same phenomenon in our control animals: the exploration of a novel empty holeboard during sub-HFS facilitated STP into LTP that lasted for at least 24 h (24 h PS, ANOVA  $F_{(1,13)} = 26.5$ ;  $P < 0.0005$ ; fEPSP, ANOVA  $F_{(1,13)} = 8.6$ ;  $P < 0.05$ ;  $n = 9$  in vehicle-treated vs.  $n = 6$  in animals exposed to an empty holeboard, Figs. 5A,B). Upon re-exposure to the same, now familiar, empty holeboard 1 week later, sub-HFS failed to elicit LTP in control animals compared with the first exposure of the animals exposed to the novel holeboard (24h: PS amplitude, ANOVA  $F_{1, 10} = 6.9$ ;  $P < 0.05$ ; fEPSP, ANOVA  $F_{1, 10} = 11.6$ ;  $P < 0.05$ ;

$n = 6$  in both groups, Figs. 5A,B). Thus, in line with previous reports (Kemp and Manahan-Vaughan, 2004), it is the novelty of the spatial experience that drives the strengthening of LTP.

When we applied the  $\beta$ -adrenoreceptor antagonist, propranolol, before sub-HFS coupled with novel empty holeboard exposure, STP was not facilitated into LTP (PS amplitude, ANOVA  $F_{(1,14)} = 0.15$ ,  $P = 0.7$ ; fEPSP, ANOVA  $F_{(1,14)} = 0.18$ ,  $P = 0.67$ ;  $n = 8$  in both groups, Figs. 6A–D). Strikingly, however, the re-exposure of the animals to the holeboard during sub-HFS, 7 days later, successfully facilitated STP into LTP (PS amplitude, ANOVA,  $F_{(1,14)} = 7.8$ ;  $P < 0.005$ ; fEPSP, ANOVA  $F_{(1,14)} = 15.4$ ;  $P < 0.05$ ;  $n = 8$  in both groups, Figs. 6A–E). This observation is in line with other reports that prevention of spatial learning prevents learning-facilitated plasticity (Lemon and Manahan-Vaughan, 2006; Popkirov and Manahan-Vaughan, 2011).



**FIGURE 5.** Learning about a novel environment facilitates long-term potentiation at perforant path-dentate gyrus synapses. (A, B) Subthreshold-high frequency stimulation (sub-HFS, three trains at 200 Hz) elicits synaptic potentiation that lasts for over 2 h, but less than 3 h, in vehicle-treated animals. Coupling sub-HFS with exploration of a novel holeboard significantly prolongs STP, resulting in a long-term potentiation (LTP) that lasts for over 25 h. Exposing the rats a second time to the now familiar holeboard does not result in LTP following sub-HFS. The STP evoked is equivalent to control responses (see Fig. 2). (C) Analog traces represent perforant path-dentate gyrus (DG) field potentials (i) 5 min before, (ii) 5 min after and (iii) 24 h after vehicle in the presence of sub-HFS, (iv) 5 min before, (v) 5 min after and (vi) 24 h after novel holeboard exploration in the presence of sub-HFS as well as (vii) 5 min before, (viii) 5 min after (ix) and 24 h after familiar holeboard exploration in the presence of sub-HFS. Vertical bar, 3 mV; horizontal bar, 2.5 ms.

### Spatial Learning of Novel Empty Space is Prevented by an Antagonist of $\beta$ -Adrenoreceptors

To assess whether treatment with the  $\beta$ -adrenoreceptor antagonist that resulted in prevention of learning-facilitated LTP, was coupled with a prevention of novel spatial learning, we monitored animal behavior during sub-HFS. One animal group received vehicle and the other received propranolol. Thirty minutes later they received sub-HFS in the presence of the novel, and 7 days later, the familiar holeboard. Typically, if

the animal remembers that it has explored a spatial environment, such as a holeboard, habituation in the form of decreased head-dipping in the holeboard holes, and less rearing behavior occurs (Lemon and Manahan-Vaughan, 2006). Here, we observed that re-exposure to the now familiar holeboard led to significantly fewer dips into, and rears above, the holes in vehicle-treated animals ( $t$ -test: rears:  $P < 0.05$  and dips:  $P < 0.05$ ; first vs. second exploration of the holeboard in  $n = 6$  animals, Figs. 7A,B). Thus, the animals remembered the spatial environment 7 days after the first holeboard exposure. However, when propranolol was given before the first exploration of the novel holeboard, evidence of habituation to the second holeboard exposure was absent ( $t$ -test, rears:  $P = 0.33$ , dips:  $P = 0.87$ , first vs. second exploration of the holeboard in  $n = 8$  animals, Figs. 7A,B). In fact, the animals behaved as if they were exploring the environment for the first time.

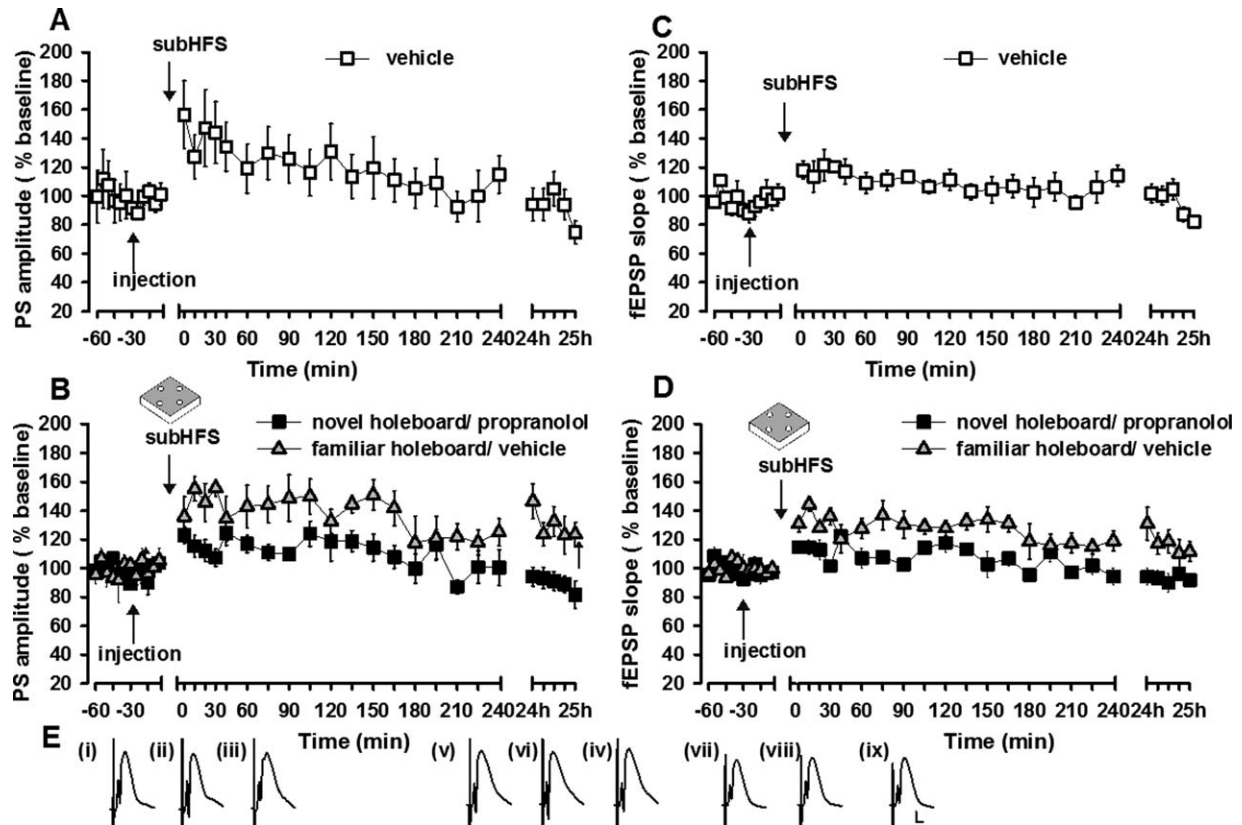
To verify that the  $\beta$ -adrenoreceptor antagonist did not elicit state-dependent changes of exploratory behavior, we compared the number of rears and dips during the first holeboard exposure in the presence of propranolol and vehicle. The number of rears and dips did not differ between groups ( $t$ -test; rears:  $P = 0.06$ , dips:  $P = 0.09$ ,  $n = 8$  propranolol vs.  $n = 6$  vehicle-treated animals).

## DISCUSSION

The results of this study support that  $\beta$ -adrenoreceptors are critically required for LTP and learning-facilitated LTP in the dentate gyrus of freely behaving rats. This may reflect the innervation by the locus coeruleus (LC) of the dentate gyrus, and the special role of the dentate gyrus as an informational filter to the hippocampus. Strikingly, LC activation that preceded high-frequency (HFS) stimulation of the perforant path (pp), significantly changed short-term potentiation (STP) into LTD, but had no effect on LTP. LC stimulation after HFS resulted in depotentiation of LTP. These findings suggest that under conditions of increased behavioural arousal, information processing and storage in the dentate gyrus is supported by LC activation and noradrenaline release acting on  $\beta$ -adrenoreceptors, such that an optimization of information storage may take place. The timing and intensity of LC activation may play a decisive role in this process.

The inhibition of dentate gyrus LTP by antagonism of  $\beta$ -adrenoreceptors was very potent, with even the induction phase of LTP being affected in both afferent electrically-induced and learning-facilitated LTP. This strong sensitivity of LTP to  $\beta$ -adrenoreceptor-regulation is also evident in the CA1 region in vivo (Kemp and Manahan-Vaughan, 2008a,2008b) but is absent in mossy fibers (Hagena and Manahan-Vaughan, 2012). Mossy fibers have molecular requirements for LTP that are very distinct to those of the dentate gyrus and CA1 region (Malenka and Nicoll, 1993; Harris and Cotmann, 1986). A common denominator for LTP at CA1 and dentate gyrus





**FIGURE 6.** The facilitation of long-term potentiation by novel spatial learning is prevented by a  $\beta$ -adrenoreceptor antagonist. (A, C) Subthreshold-high frequency stimulation (sub-HFS, three trains at 200 Hz) elicits STP that last for less than 3 h in vehicle-treated animals. Prior treatment with the  $\beta$ -adrenoreceptor antagonist propranolol (2  $\mu$ g) inhibits the facilitation of LTP by exposure to a novel holeboard that typically occurs in vehicle-treated controls (see Fig. 3). In contrast exposing the rats a second time to the now familiar holeboard facilitates LTP (B, D). This suggests that

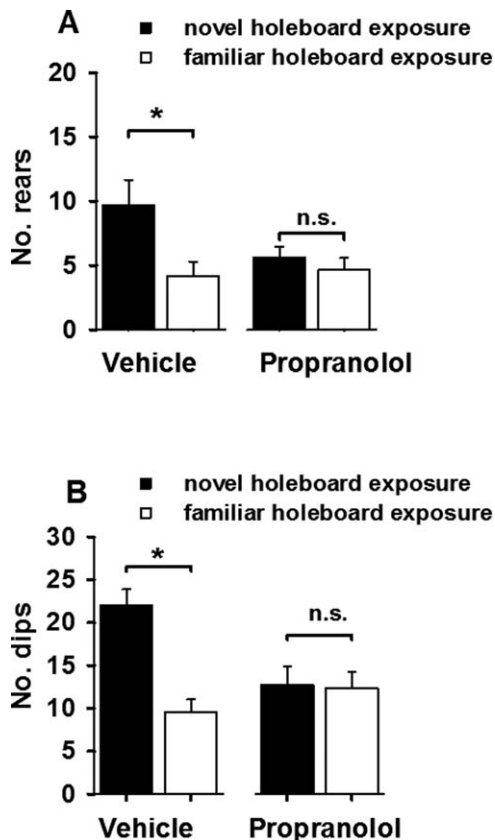
propranolol prevented spatial learning in conjunction with LTP (see Fig. 7). (E) Analog traces represent perforant path-dentate gyrus (DG) field potentials (i) 5 min before, (ii) 5 min after, and (iii) 24 h after vehicle in the presence of sHFS, (iv) 5 min before, (v) 5 min after, and (vi) 24 h after propranolol in the presence of sHFS coupled with the novel holeboard exposure and (vii) 5 min before, (viii) 5 min after, and (ix) 24 h after vehicle in the presence of sHFS coupled with the second holeboard exposure. Vertical bar, 3 mV; horizontal bar, 2.5 ms.

synapses comprises activation of the *N*-methyl-D-aspartate (NMDA) receptor (Malenka and Nicoll, 1993). Activation of  $\beta$ -adrenoreceptors increases  $Ca^{2+}$  influx through NMDA receptors (Raman et al., 1996). The dramatic suppression of initial potentiation immediately following HFS in the presence of the  $\beta$ -adrenoreceptor antagonist in the present study, suggests that this regulation is highly important for NMDA receptor-dependent LTP.  $\beta$ -adrenoreceptor antagonism blocks granule cell depolarization (Stanton et al., 1989), and  $\beta$ -adrenoreceptors modulate voltage-dependent calcium channels in granule cells (Gray and Johnston, 1987). Thus, these receptors also regulate excitability that can act permissively towards the induction of synaptic plasticity.

The strengthening of STP into (>24 h) LTP indicates that this receptor also supports the downstream biochemical processes required for persistent LTP. The cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) cascade plays a pivotal role in late LTP (Stanton and Sarvey, 1985; O'Dell et al., 2010) and is stimulated by activation of  $\beta$ -

adrenoreceptors. Protein synthesis that underlie very persistent forms of LTP also requires  $\beta$ -adrenoreceptor activation in vitro (Gelinias and Nguyen, 2005) and in vivo (Straube and Frey, 2003), but this dependency on  $\beta$ -adrenoreceptors can be overcome by very strong afferent stimulation protocols (Straube and Frey, 2003). Further in vitro experiments also confirm that  $\beta$ -adrenoreceptor activation is essential to convert STP into late LTP (Connor et al., 2012). Taken together, our data indicate that under circumstances where information processing results in less robust storage (i.e. weak LTP or STP) the activation of  $\beta$ -adrenoreceptors will serve to reinforce this information and presumably generate a more robust and longer lasting engram of the stored experience.

This property may be tightly dependent on the timing and degree of noradrenaline release from the LC. In line with this possibility, is the finding that LC activation before afferent stimulation of the perforant path (pp) serves to eliminate weak LTP, to such an extent that LTD results. Robust LTP (>24 h) is impervious to the same kind of (prior) LC stimulation, but



**FIGURE 7.** Novel spatial learning is prevented by a  $\beta$ -adrenoreceptor antagonist. The bar charts illustrate the mean number of rears in (A) or dippers in (B) (nose-pokes into holeboard holes) that occurred when the animals were exposed to the holeboard. The mean number of rears (A) and dippers (B) is significantly lower when performance during holeboard re-exposure (white bars) is compared with performance during novel exposure (black bars). Treatment with propranolol resulted in equivalent rearing (A) and dipping (B) behavior during novel and familiar holeboard exposure. Performance was equivalent to the behaviour of vehicle-treated animals during novel holeboard exposure. This confirms that propranolol prevented learning of the spatial environment. *t*-test: \* $P < 0.05$ .

LC activation that occurs subsequent to HFS depotentiates LTP. The concentrations of  $\beta$ -adrenoreceptor agonist and antagonist used in the present study were subthreshold for effects on basal synaptic transmission. Thus, no effects on  $\beta$ -adrenoreceptor-mediated basal tonus occurred. The strengthening of STP into LTP and curtailment of LTP into STP elicited by the agonist and antagonist, respectively, suggest that a threshold level of NA release and  $\beta$ -adrenoreceptor activation is prerequisite for the induction of robust LTP. This likelihood has been confirmed by several *in vivo* (Straube and Frey, 2003) and *in vitro* studies (Gelinan and Nguyen, 2005). Under certain circumstances, the LC system may “reset” the DG synapses to a “naive” level to be ready to store novel information, however (Bouret and Sara, 2005; Aston-Jones and Cohen, 2005; Sara and Bouret, 2012). Our finding that weak LTP is prevented by prior LC stimulation may correspond to this property.

The stimulation protocol we used to activate the LC is arguably quite a strong one. It results in hippocampal LTD that is dependent on both  $\beta$ -adrenoreceptor and dopamine D1-receptors (Lemon and Manahan-Vaughan, 2011), and triggers potent elevations of NA and discrete elevations of dopamine in the CA1 region (Lemon et al, 2009); an area that does not receive direct LC innervation. Strong activation of the LC results in co-release of noradrenaline and its precursor dopamine (Smith and Green, 2012). Weak, but not strong, LTP critically depends on activation of dopamine D2 receptors (Manahan-Vaughan and Kulla, 2003), and activation of either dopamine D1 or D2 receptors prevents DG depotentiation (Kulla and Manahan-Vaughan, 2000; Manahan-Vaughan and Kulla, 2003). Taken together this suggests that the strong regulation of LTP processes by LC activation may relate on the one hand to activation of  $\beta$ -adrenoreceptors, and on the other, on activation of dopamine receptors, that may result from noradrenaline and dopamine co-release, respectively. The degree of co-release of dopamine, in addition to noradrenaline can be expected to be determined by the relative degree of arousal of the animal and the behavioural saliency of the novel spatial experience.

The regulation by  $\beta$ -adrenoreceptors of synaptic plasticity in the different hippocampal subfields is not homogenous. In Schaffer collateral-CA1 and perforant path-dentate gyrus synapses, HFS-induced LTP, learning-facilitated LTP (Kemp and Manahan-Vaughan, 2008a,2008b), and LTP that is strengthened by novelty (Straube et al., 2003) is dependent on  $\beta$ -adrenoreceptors, whereas in mossy fiber-CA3 synapses, only learning-facilitated LTP is dependent on the activation of  $\beta$ -adrenoreceptors (Hagena and Manahan-Vaughan, 2012). In the dentate gyrus, agonist activation of  $\beta$ -adrenoreceptors strengthens LTP (data of the present study), whereas this effect is absent in the CA1 region *in vivo* (Kemp and Manahan-Vaughan, 2008a,2008b). The pronounced effect of noradrenaline on LTP in the dentate gyrus, compared with other hippocampal subregions, may be related to the fact that the noradrenergic innervation originating from the LC is very dense in the dentate gyrus and noradrenaline content in this subregion is prominent (Loy et al., 1980; Fallon and Loughlin, 1987). The CA1 and CA3 regions receive fewer noradrenergic projections from the LC than the dentate gyrus (Loy et al., 1980). Additionally,  $\beta$ 1- and  $\beta$ 2-adrenoreceptors are not equally distributed in the rat hippocampus: the highest expression of  $\beta$ 1- and  $\beta$ 2-adrenoreceptors is found in the dentate gyrus (Milner et al., 2000), whereas lower receptor densities occur in the CA1, and the lowest receptor densities occur in the CA3 region (Booze et al., 1993). The distribution pattern of  $\beta$ 1- and  $\beta$ 2-adrenoreceptors may thus explain why the neuromodulatory effect mediated by  $\beta$ -adrenoreceptors is most prominent in the dentate gyrus compared with the CA subfields studied thus far.

The tight regulation by the noradrenergic system of synaptic plasticity in the dentate gyrus may serve to support its role as a structure that adapts flexibly to incoming information, and responds best to salient information. Incoming information may be conferred with a “weighting” according to saliency or

novelty, that is mediated by  $\beta$ -adrenoreceptor activation (Lemon et al., 2009), thereby enabling the dentate gyrus to engage in “context reset” as postulated for this structure in the encoding of episodic memory function (Cheng et al., 2013). In line with this, we observed that weak synaptic potentiation is facilitated into LTP in the dentate gyrus by a novel spatial learning experience, and that this facilitation is prevented by antagonism of  $\beta$ -adrenoreceptors. The antagonism of  $\beta$ -adrenoreceptors also affects the early phase of LTP, as does prior LC stimulation. LTD that was elicited by coupling test-pulse pp-stimulation with LC stimulation also became apparent immediately after the conclusion of activation. This suggests that noradrenaline release from the LC that acts on  $\beta$ -adrenoreceptors is very rapid, and its influence on the direction of change in synaptic strength is dependent on the ambient and momentary noradrenaline tonus.

The regulation by  $\beta$ -adrenoreceptors of synaptic plasticity is common to all hippocampal synapses (Kemp and Manahan-Vaughan, 2008a,2008b; Hagena and Manahan-Vaughan, 2012) but what is distinct to the dentate gyrus synapse is that agonist activation of  $\beta$ -adrenoreceptors also enhances STP into LTP, whereas in the CA1 region, for example it does not (Kemp and Manahan-Vaughan, 2008a,2008b). In mossy fiber synapses, antagonism of  $\beta$ -adrenoreceptors also does not prevent LTP that is induced by HFS (Hagena and Manahan-Vaughan, 2012). Taken together, this suggests that the dentate gyrus is much more sensitive to  $\beta$ -adrenergic regulation and to subtle changes in the level of LC noradrenaline release than the other hippocampal subfields.

The beta-adrenergic modulation of synaptic plasticity in the dentate gyrus allows neocortical inputs to the dentate gyrus to be filtered and prioritised based on novelty or saliency. Information reaching the CA3 region from the dentate gyrus is subsequently evaluated in a match-mismatch process whereby the CA3 region is believed to compare internal representations with incoming sensory information from the environment (Lee et al., 2005). It is likely that noradrenergic neuromodulation of synaptic plasticity contributes to this match-mismatch process. The dentate gyrus, with its great sensitivity to noradrenergic modulation, may serve to increase the signal-to-noise ratio of incoming information such that the processing and storage of novel information is prioritised (Lemon et al., 2009; Lemon and Manahan-Vaughan, 2012) and subsequent information processing by subfields such as the CA3 region is optimised.

The exploration of a novel spatial environment leads to LTP facilitation in the DG (Kemp and Manahan-Vaughan, 2008a,2008b; Straube et al., 2003) and is dependent on the activation of  $\beta$ -adrenoreceptors and protein synthesis (Straube et al., 2003). Our observation that spatial learning is prevented by  $\beta$ -adrenoreceptor antagonism, is in line with previous reports that the encoding of spatial memory that is enhanced by LC activation can be blocked by  $\beta$ -adrenoreceptor antagonism (Lemon et al., 2009). LC neurons increase firing after novelty exposure (Sara et al., 1994; Vankov et al., 1995; Kitchingina et al., 2013b) and LC-induced learning is  $\beta$ -adrenoreceptor-dependent (Devauges and Sara, 1991; Sara

et al., 1994). The hippocampal locus for detecting “novelty”-elicited changes is not fully understood; some propose the CA1 and CA3 regions as being key to this process (Vianna et al., 2000; Lee et al., 2004), whereas others favor the dentate gyrus as the responsible subregion (Straube et al., 2003). Our data agree with reports by others (Lee et al., 2005; Hunsacker et al., 2008; Penley et al., 2013) that propose that the dentate gyrus is a pivotal structure in the processing of novelty detection by the hippocampus.

LTP is not the only means through which spatial experience is encoded by the hippocampus (Kemp and Manahan-Vaughan, 2007). LTD expression in the dentate gyrus and CA1 region in vivo is facilitated by LC stimulation and this process requires activation of  $\beta$ -adrenoreceptors (Lemon et al., 2009; Hansen and Manahan-Vaughan, 2014). How can this finding be reconciled with the fact that  $\beta$ -adrenoreceptors are also required for learning-facilitated LTP? LTD and LTP are likely to encode different aspects of a spatial representation (Kemp and Manahan-Vaughan, 2007), and rely on relative and plasticity-specific changes in postsynaptic activity and intracellular  $Ca^{2+}$  elevations (Lisman, 2001). LTP is associated with the encoding of context-dependent fear memory (Nabavi et al., 2014), global changes in space/scene changes (Kemp and Manahan-Vaughan, 2004, 2007) and spatial novelty (Straube et al., 2003). LTD is associated with learning about specific aspects of spatial content (Kemp and Manahan-Vaughan, 2004; Hagena and Manahan-Vaughan, 2011; Goh and Manahan-Vaughan, 2013). Thus, depending on the nature and the novelty of the spatial information to be encoded, noradrenaline release onto the hippocampus, that acts on  $\beta$ -adrenoreceptors can specifically support information encoding by LTP and LTD.

## CONCLUSIONS

The activation of  $\beta$ -adrenoreceptors is crucial for LTP in the dentate gyrus that is induced either by HFS, or that occurs in association with spatial learning. Dentate gyrus LTP, that is facilitated by appetitive stimuli is also  $\beta$ -adrenoreceptor-dependent (Seidenbecher et al., 1997), as is dentate gyrus LTD that is associated with novel spatial experience (Kemp and Manahan-Vaughan, 2008a,2008b) or by LC stimulation (Hansen and Manahan-Vaughan, 2014). This suggests that the locus coeruleus, when activated by novel experience (Sara et al., 1994; Vankov et al., 1995; Kitchingina et al., 1997), results in noradrenergic activation of hippocampal  $\beta$ -adrenoreceptors that promotes encoding of novel experience by means of synaptic plasticity. Strikingly, we observed that in contrast to hippocampal LTD that is enabled by LC stimulation, direct activation of the LC before HFS, converted weak forms of potentiation into LTD and had no effect on robust LTP. LC stimulation after HFS caused depotentiation of robust LTP. This suggests that the degree and pattern of LC stimulation, and resulting release



of noradrenaline in the hippocampus, may serve to sculpt synaptic encoding that subserves long-term spatial memory. Dopamine release from the locus coeruleus (Lemon et al, 2009; Smith and Green, 2012) may contribute importantly to this kind of regulation. Taken together these data suggest that the LC and hippocampal  $\beta$ -adrenoreceptors play a pivotal role in the fine-tuning of synaptic information storage that underlies spatial memory.

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