Locus coeruleus stimulation and noradrenergic modulation of hippocampo-prefrontal cortex long-term potentiation

Ee Peng Lim1,2, Chay Hoon Tan1, Thérese M. Jay3,4 and Gavin S. Dawe1,2

1 Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
2 Neurobiology and Ageing Programme, Life Sciences Institute, National University of Singapore, Singapore
3 INSERM, U894, Physiopathologie des Maladies Psychiatriques, Centre de Psychiatrie et Neurosciences, Paris, France
4 Université Paris Descartes, Faculté de Médecine Paris Descartes, Paris, France

Abstract

Stimulation of the subiculum/CA1 of the hippocampal formation evokes monosynaptic field potentials in the prefrontal cortex (PFC). High-frequency stimulation of the hippocampus (HPC) can induce long-term potentiation (LTP) in this hippocampo-prefrontal cortical (hippo-PFC) pathway. Previous studies have shown that dopamine and serotonin modulate hippo-PFC LTP. Here, we investigated whether the locus coeruleus (LC) and noradrenaline (NA) can modulate LTP in the rat hippo-PFC pathway. Stimulation of the LC in combination with stimulation of the HPC increased hippo-PFC LTP. Infusion of lidocaine into the LC reduced hippo-PFC LTP. Administration of the noradrenaline reuptake inhibitor, nisoxetine or the \(\alpha_2\) adrenoceptor antagonist, idazoxan prior to high-frequency stimulation of the HPC enhanced hippo-LTP. In contrast, administration of clonidine, an \(\alpha_2\) adrenoceptor agonist, impaired hippo-PFC LTP. Partial noradrenergic (NAergic) lesioning with DSP-4 also impaired hippo-PFC LTP. In conclusion, the LC and NAergic mechanisms modulate hippo-PFC LTP.

Received 23 April 2009; Reviewed 2 June 2009; Revised 24 October 2009; Accepted 16 November 2009; First published online 4 February 2010

Key words: Hippocampus, noradrenaline, prefrontal cortex, synaptic plasticity.

Introduction

The locus coeruleus (LC) innervates many regions of the brain and modulates cognitive functions through the release of noradrenaline (NA) (Sara, 2009). Both prefrontal cortex (PFC) (Lewis & Morrison, 1989) and hippocampus (HPC) (Loy et al. 1980) have a relatively rich noradrenergic (NAergic) innervation originating from the LC. It is believed that projections from the LC to the PFC and HPC modulate cognitive functions, including working memory. In these regions, NA influences physiological, behavioural and cognitive responses by modulating inhibitory and excitatory synaptic transmission as well as other neuronal processes.

The PFC has been implicated in many cognitive and executive functions including arousal, attention and working memory (Dalley et al. 2004). Many neurotransmitters and neuromodulators [e.g. dopamine (DA), serotonin (5-HT), acetylcholine, NA] contribute to normal PFC cognitive functioning (Robbins & Arnsten, 2002). NA has consistently been shown to be important for working memory in animals and human (Chamberlain et al. 2006). Substantial evidence indicates that NA exerts a potent modulatory influence on PFC function through actions at adrenoceptors, with actions at \(\alpha_4\) adrenoceptors probably playing a role in working memory while \(\beta\) adrenoceptors are more likely to be involved in long-term memory (Ramos & Arnsten, 2007).

The HPC and PFC, linked by the hippocampus-prefrontal cortical (hippo-PFC) pathway, participate in various cognitive functions (Eichenbaum, 2004; Passingham & Sakai, 2004). In recent years, there has been increasing interest in the implications of the interactions between the HPC and PFC for...
cognitive functions such as memory consolidation, episodic memory and goal-directed behaviour (Ferbinteanu et al. 2006; Kesner & Rogers, 2004; Laroche et al. 2000; Simons & Spiers, 2003). There is a direct projection from the CA1 and subicular regions of the HPC to the PFC and it is reported that the pathway can undergo long-term potentiation (LTP) in vivo (Ferin et al. 1987; Jay et al. 1989; Laroche et al. 1990). Animal studies have implicated the hippo-PFC pathway in working memory, a PFC-dependent cognitive function (Floresco et al. 1997; Wang & Cai, 2006).

Evidence shows that DA is important in the induction and maintenance of LTP in hippo-PFC synapses (Gurden et al. 1999, 2000). 5-HT has also been implicated in hippo-PFC synaptic plasticity (Ohashi et al. 2002, 2003). Since dopaminergic, serotonergic and NAergic receptors are found abundantly in the PFC (Goldman-Rakic et al. 1990) and are involved in PFC-dependent cognitive behaviours (Robbins, 2005), it is possible that NA may also play a role in hippo-PFC plasticity. Currently we do not know how manipulation of the NAergic system would affect the hippo-PFC pathway.

To better understand the role of NA on hippocampal input to the PFC in vivo, we attempted to study the effects of endogenous and exogenous NA on the pathway response. We first studied the effects of enhancing NA release via LC stimulation. Many studies have shown that LC stimulation increases NA release in the terminal regions (Florin-Lechner et al. 1996). Next, we looked at the effects of enhancing and reducing NA on hippo-PFC LTP with systemic administration of nisoxetine (a NAergic reuptake inhibitor) and clonidine (an α2 adrenoceptor agonist), respectively. We also studied the effect of a partial NAergic lesion in rats pre-treated by systemic administration of DSP-4. DSP-4 binds to NA re-uptake sites resulting in degeneration of the terminals and is specific to neurons whose cell body lie in the LC (Lyons et al. 1989). Finally, we investigated the effects of enhancing NA release with idazoxan, an α2 adrenoceptor antagonist, on hippo-PFC LTP in the freely moving rat.

As dysfunctions of the HPC and PFC have been implicated in the cognitive impairments seen in autism (Loveland et al. 2008), ageing (Grady et al. 2003), depression (Goldapple et al. 2004) and schizophrenia (Harrison, 1999; Meyer-Lindenberg et al. 2005), investigation of the mechanisms of modulation of hippo-PFC LTP may suggest new therapeutic approaches to amelioration of cognitive dysfunctions in psychiatric disorders.

Materials and methods

Animals

Adult male Sprague–Dawley rats (270–320 g) (CARE, National University of Singapore) were group-housed (n = 4–6 per cage) in a temperature-controlled (22 °C) colony room with food and water available ad libitum and maintained on a 12-h light/dark cycle (lights on 07:00 hours). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore and were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Guide for the Care and Use of Laboratory Animals, National Institute of Health, USA.

Electrophysiological procedures

Rats were anaesthetized with chloral hydrate (400 mg/kg) and mounted in a stereotaxic frame with body temperature maintained at 37 ± 0.5 °C by a homeothermic blanket. The procedures for implanting electrodes and recording extracellular field potentials in the prelimbic area of the PFC were adapted from those previously described (Gurden et al. 2000; Laroche et al. 1990). A concentric bipolar stainless-steel stimulating electrode (250 μm diameter with a tip separation of 500 μm) was placed in the CA1/subicular region of the ventral hippocampal formation (6.3 mm posterior and 5.5 mm lateral to bregma). A monopolar extracellular stainless-steel recording electrode (100 μm diameter) was lowered into the ipsilateral prelimbic area of the medial PFC (3.3 mm anterior and 0.9 mm lateral to bregma) according to the atlas of Paxinos & Watson (1997). Stimulation of the CA1/subicular region evokes a characteristic monosynaptic negative-going field potential with peak latency of 18–21 ms. The depths of the recording and stimulating electrodes (2.5–4.0 mm and 5.0–6.4 mm below the cortical surface, respectively; Fig. 1a) were adjusted to maximize the amplitude of the negative-going wave of the field excitatory postsynaptic potential (PSP). During localization of the response, stimulation (250 μs pulse width) was delivered once every 10 s at 250 μA. Once electrode placement was complete, a period of 30 min was allowed for stabilization of responses. An input/output characterization was then established for each rat.

Stimulation (250 μs pulse width) was delivered once every 10 s (S8800, Grass-Telefactor, USA). If >500 μA was required to evoke a maximal response the animal was excluded from the study as stimulation at such...
high intensities would probably result in damage. Test pulses (250 μs pulse width) were delivered once every 30 s at an intensity that evoked a 50% maximal response. Baseline recording was then conducted for 20 min. LTP was produced by one or two series (6 min apart) of high-frequency stimulation (HFS) (10 trains, 250 pulses/s, 250 ms duration delivered at 0.1 train/s) in the HPC at a stimulus intensity that evoked a 70% maximal response. After HFS, baseline stimulation was resumed and recording was continued for at least 60 min. For recording in freely moving animals, some animals were chronically implanted with stainless-steel electrodes (Plastics One Inc., USA) under ketamine-xylazine anaesthesia. The procedure was similar to the acute experiment except that the electrodes were fixed to the skull with dental acrylic and connected to a commutator for recording while the animal was awake following a recovery period of at least 7 d. Responses were recorded in these animals for at least 210 min.

Field potentials were amplified and bandpass-filtered 0.1–3 kHz (2400A, Dagan Corporation, USA) and 50 Hz noise was subtracted (HumBug, Quest Scientific, Canada) before digitization at 25 kHz [Micro 1401, Cambridge Electronic Design (CED), UK]. Data collection and stimulus delivery were under computer control with Signal software (CED). Data were also analysed with the Signal software and PSP amplitudes were expressed as the percentage change from the baseline.

**LC stimulation**

In some experiments, the ipsilateral LC was stimulated. Bipolar stimulating electrodes were constructed from two twisted wires (50 μm diameter) which were insulated except for the cut ends. During electrode placement, unit activity was recorded with one pole of the electrode. The electrode was positioned above the LC (9.8 mm posterior and 1.1–1.3 mm lateral to bregma) and lowered until LC neurons were reached (5–6 mm below the cortical surface). Signals were amplified and bandpass-filtered from 0.3 to 3 kHz (2400A, Dagan Corporation), digitized at 25 kHz (Micro 1401, CED), and neuronal activity was monitored with Spike2 software (CED) and computer speakers. LC neurons were identified by their characteristic action potential waveform, firing rate and biphasic response to hindpaw pinch, as previously described (Dawe et al. 2001). Once activity characteristic of the LC was identified, electrodes were held in place with dental cement. Only animals in which correct placement of the stimulating electrode in the LC was confirmed by subsequent histological examination were included in the analysis. The wires were disconnected from the preamplifier and connected to a stimulus generator (S8800, Grass-Telefactor) and the stimuli were delivered through a DC constant-current generator isolation unit (Grass-Telefactor). The LC was stimulated using a regularly spaced pattern of pulses presented at 12 Hz (350 μA, 200 μs duration). This stimulation protocol was selected because a similar one was previously reported to enhance NA release in the PFC (Florin-Lechner et al. 1996). The effect of LC stimulation on hippo-PFC LTP was
studied in a group of four rats where 12 Hz stimulation (8.25 s) was applied to the LC prior to each of the 10 trains of stimuli to the HPC necessary to induce LTP (see above for protocol). These rats were compared with a group of rats \( (n = 5) \) in which one series of HFS was applied alone to induce LTP in the hippo-PFC pathway.

**LC infusion**

The LC was temporarily inactivated by microinjection of 4% lidocaine hydrochloride solution (Sigma-Aldrich, USA). It has been shown that 1–4% lidocaine is necessary to inactivate brain tissue (Tehovnik & Sommer, 1997). An implantation cannula (Plastics One Inc.) was stereotaxically placed above the LC and lowered at the same time as the electrophysiological electrodes. A dummy cannula was then placed inside the implantation cannula. Five minutes before infusion, the dummy cannula was removed and replaced with an injection needle (30-gauge) connected to a short piece of polyethylene tubing and a 1 μl Hamilton syringe. The needle was inserted 1 mm beyond the tip of the cannula and was placed just above the LC to prevent mechanical damage to the LC. Five minutes before HFS, 0.2 μl saline or lidocaine were injected over 25 s (Micro4 microsyringe pump controller, World Precision Instruments Inc., USA). The needle was left in place for another 60 s before it was slowly withdrawn. The position of the cannula was assessed histologically for each rat by 30 μm coronal sectioning and staining with Cresyl Violet.

**Drugs**

NAergic drugs were administered intraperitoneally (i.p.) after 20–30 min baseline recording. The rats received either 1 mg/kg nisoxetine HCl (Tocris Bioscience, UK) \( (n = 4) \), 1 mg/kg clonidine HCl (Tocris Bioscience) \( (n = 5) \), or saline \( (0.9 \% \text{ NaCl}) \) \( (n = 6) \). Then 4 mg/kg idazoxan (Sigma-Aldrich) \( (n = 4) \) was used in the freely moving experiment. Drugs were prepared on the day of the experiment. Post-injection recordings were conducted for another 20–30 min. LTP was then induced using two series of HFS as described above. At the end of the recording, the rats were perfusion-fixed as described below. All data presented are for the vehicle-treated control group (mean ± S.E.M.).

**Histological verification**

**Hippo-PFC recording/stimulation site**

Following each experiment, the rats were perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4)
LC stimulation site

To visualize LC stimulation sites the LC was electrolytically lesioned and rats were perfused with 4% paraformaldehyde (Sigma-Aldrich) containing 5% potassium ferrocyanide (Sigma-Aldrich) to produce a Prussian Blue reaction product marking iron deposited from the stimulating electrode tip. Frozen 30 μm coronal sections were cut through the pons and counterstained with Nuclear Fast Red. Only data from rats which had electrode placements in the LC were used (see Fig. 1b).

Data analysis and statistics

Data collection and analysis were performed with Signal software (CED). Statistical analysis was performed with SPSS software (SPSS Inc., USA). PSP amplitude was expressed as a percentage of the mean amplitude during the baseline recording. All results are given as mean ± S.E.M. Electrophysiological data were averaged in consecutive 20- to 30-min periods after LTP induction and analysed using repeated-measures ANOVA. Depending on the experiment, either one-way ANOVA or independent-sample t tests were then used to discriminate effects of manipulations for each time period. Significant ANOVA results were followed-up with post-hoc Tukey’s HSD comparison. All statistical tests were applied with a two-tailed significance criterion of p < 0.05.

Results

LC stimulation enhanced LTP in the rat hippo-PFC pathway

In experiments involving LC stimulation, only animals in which the electrode was histologically verified as being placed in the LC were included in the analysis. The position of the steel LC stimulating electrode was identified by production of a small electrolytic lesion and subsequent histological processing for the Prussian Blue reaction after the recording (Fig. 1b).

One series of HFS to the HPC produced LTP in the hippo-PFC pathway. This LTP was characterized by a significant increase in the amplitude of the evoked response (Fig. 2b). When stimulation of the LC was applied prior to each of the 10 trains of stimuli used in the hippocampal tetanization protocol, LTP of the PFC PSP was further potentiated (group effect: $F_{1,2} = 7.497, p < 0.05$). As shown in Fig. 2c, the enhancement of LTP produced by LC stimulation was statistically significant for the first two 20-min epochs compared to the group receiving only a single HPC HFS ($T_{19} = 1.44, F_{19.20} = 1.359, p < 0.05$; group effect: $F_{19,19} = 1.359, p < 0.05$; $T_{19.20} = 1.359, F_{19.20} = 1.359, p < 0.05$; $T_{30.30} = 1.359, F_{30.30} = 1.359, p < 0.05$; $T_{40.40} = 1.359, F_{40.40} = 1.359, p < 0.05$; $T_{50.50} = 1.359, F_{50.50} = 1.359, p < 0.05$).

Systemic NAergic drugs influenced LTP in the rat hippo-PFC pathway

Various NAergic drugs were systemically administered 20 min prior to two series of HFS of the HPC. Overall, NAergic drugs have a significant effect on LTP in the hippo-PFC pathway (time effect: $F_{4,44} = 135.431, p < 0.0001$; time x group effect: $F_{4,44} = 23.143, p < 0.0001$; group effect: $F_{2,11} = 25.928, p < 0.0001$; Fig. 4b).

Infusion of local anaesthetic into the LC reduced LTP in the rat hippo-PFC pathway

Saline or 4% lidocaine (0.2 μl) was infused at 0.4 μl/min into the LC 5 min prior to two series of HFS of the HPC. Infusion of lidocaine had a significant effect on LTP in the hippo-PFC pathway (time effect: $F_{1,2} = 40.364, p < 0.0001$; time x group effect: $F_{1,2} = 5.315, p < 0.0005$; group effect: $F_{1,2} = 14.300, p < 0.05$; Fig. 3b). As shown in Fig. 3c, the impairment of LTP produced by lidocaine was also statistically significant over 60 min compared to the saline-treated group (saline vs. lidocaine, $T_{0-10} = 190.9 ± 6.2%$ vs. $140.6 ± 10.5%, p < 0.05$; $T_{10-20} = 197.3 ± 6.9%$ vs. $144.0 ± 16.2%, p < 0.05$; $T_{20-30} = 199.0 ± 18.2%$ vs. $143.7 ± 9.6%, p < 0.05$; $T_{30-40} = 181.5 ± 13.2%$ vs. $144.3 ± 3.2%, p < 0.05$; $T_{40-50} = 187.4 ± 10.1%$ vs. $140.4 ± 8.6%, p < 0.05$; $T_{50-60} = 173.3 ± 11.8%$ vs. $131.1 ± 6.3%, p < 0.05$).

Noradrenergic and LC modulation of hippo-PFC LTP

(Sigma-Aldrich). Brains were removed and sectioned. Coronal sections were stained with Nuclear Fast Red to visualize the electrode tracks.
Group effects were also significant at various time-points following induction of LTP ($T_{0-20}$ min: $F_{2,13} = 30.450$, $p < 0.0001$; $T_{20-40}$ min: $F_{2,13} = 24.517$, $p < 0.0001$; $T_{40-60}$ min: $F_{2,13} = 22.636$, $p < 0.0001$). As shown in Fig. 4c, enhancing endogenous NAergic activity with a noradrenergic re-uptake inhibitor, nisoxetine (1 mg/kg i.p.) augmented hippo-PFC LTP (saline vs. nisoxetine, $T_{0-20}$ min: 177.6 ± 9.4% vs. 227.3 ± 14.8%, $p < 0.05$; $T_{20-40}$ min: 182.3 ± 9.9% vs. 216.1 ± 16.0%, n.s.; $T_{40-60}$ min: 168.7 ± 10.8% vs. 212.4 ± 15.6%, $p < 0.05$). The reverse was true when we inactivated the LC with an $\alpha_2$ adrenoceptor agonist. Clonidine (1 mg/kg i.p.) impaired LTP over a period of 60 min after tetanic stimulation (saline vs. clonidine, $T_{0-20}$ min: 177.6 ± 9.4% vs. 112.9 ± 7.0%, $p < 0.01$; $T_{20-40}$ min: 182.3 ± 9.9% vs. 115.1 ± 4.4%, $p < 0.01$; $T_{40-60}$ min: 168.7 ± 10.8% vs. 110 ± 5.4%, $p < 0.01$; Fig. 4c). Clonidine did not completely abolish LTP and induction of a stronger LTP with three series of HFS to the HPC could largely overcome the suppression induced by clonidine [Fig. S3 (online)].

**NAergic lesion with DSP-4 impaired LTP in the rat hippo-PFC pathway**

Next, we examined the effects of cortical NAergic depletion on changes in LTP at the hippo-PFC synapses. When LTP (two series of HFS) was induced in DSP-4-treated rats ($n = 7$), amplitude of the evoked response was greatly reduced compared to vehicle-treated rats ($n = 5$) (group effect: $F_{1,13} = 5.894$, $p < 0.05$; Fig. 5b). As shown in Fig. 5c, the impairment of LTP produced by DSP-4 lesion was also statistically significant for the first 40 min compared to the vehicle-treated group (vehicle vs. DSP-4, $T_{0-20}$ min: 177.6 ± 9.4% vs. 153.5 ± 5.1%, $p < 0.05$; $T_{20-40}$ min: 182.3 ± 9.9% vs. 153.9 ± 4.5%, $p < 0.05$; $T_{40-60}$ min: 168.7 ± 10.8% vs. 147.2 ± 4.7%, n.s.).

The lesions of NAergic terminals at the PFC were confirmed by staining for D2/JH immunoreactivity at 1 wk after lesions were induced by DSP-4 (50 mg/kg). As shown in Fig. 5(d, e), D2/JH immunoreactivity was markedly reduced in DSP-4-treated rats compared to vehicle-treated rats. The number of D2/JH-immunopositive fibres in the PFC of DSP-4-lesioned rats was reduced by 84.4% compared to the vehicle-treated control group.
Activation of NAergic activity with idazoxan, an \( \alpha_2 \) adrenoceptor antagonist, enhanced hippo-PFC LTP in the freely moving rat

As the inactivation of NA activity with an \( \alpha_2 \) adrenoceptor agonist impaired hippo-PFC LTP (see above), we postulated that \( \alpha_2 \) adrenoceptor antagonism would do the reverse. The effects of activating NAergic activity with an \( \alpha_2 \) adrenoceptor antagonist on hippo-PFC LTP were studied in the freely moving rat to examine if the NAergic effect could be observed in the absence of anaesthesia. Indeed, idazoxan (4 mg/kg i.p.) enhanced LTP after HFS (\( n = 4 \)) (Fig. 6b). As shown in Fig. 6c, the augmentation of LTP produced by idazoxan in the first 90 min was statistically significant compared to the saline-injected group (saline vs. idazoxan, \( T_{0-30 \text{ min}} : 142.4 \pm 9.7\% \) vs. 194.2 \pm 18.8\%, \( p < 0.05 \); \( T_{30-60 \text{ min}} : 147.9 \pm 7.8\% \) vs. 186.3 \pm 13.4\%, \( p < 0.05 \); \( T_{60-90 \text{ min}} : 140.9 \pm 8.0\% \) vs. 175.5 \pm 11.0\%, \( p < 0.05 \); \( T_{90-120 \text{ min}} : 138.4 \pm 9.2\% \) vs. 162.3 \pm 7.0\%, n.s.; \( T_{120-150 \text{ min}} : 134.1 \pm 9.3\% \) vs. 159.5 \pm 14.0\%, n.s.).

Another \( \alpha_2 \) adrenoceptor antagonist, yohimbine, also produced a comparable enhancement of hippo-PFC LTP in the anaesthetized rat [Fig. S4 (online)].

Discussion

In this study we first showed that electrical stimulation of the LC, using a stimulation protocol similar to that previously shown to release NA in the PFC, can enhance synaptic plasticity of the rat hippo-PFC pathway \textit{in vivo}. We also showed that LTP in this pathway may be affected by pharmacological agents that modulate the NAergic system when systemically administered. Our data show that treatments which increase NA release enhanced hippo-PFC LTP, while those which decrease NA had the opposite effect. These results suggest that the NAergic system may modulate synaptic plasticity at the hippo-PFC synapse \textit{in vivo}.

When we paired single hippocampal tetanus together with regularly spaced 12 Hz LC stimulation, the post-tetanic response in the PFC was enhanced compared to single hippocampal tetanus alone. A previous study demonstrated that a 10-Hz tonic LC stimulation protocol produced a 66\% increase in over different time periods. The columns represent mean \( \pm \) S.E.M. of the average normalized PSP amplitude in consecutive 10-min periods before and after HFS. (* \( p < 0.05 \) compared to saline-infused group). LC + saline, group receiving saline infusion into the LC; LC + lidocaine, group receiving lidocaine infusion into the LC; Inf, infusion.
NA release in the PFC and that this the elevation lasted for about 40 min (Florin-Lechner et al. 1996). These results imply that NA may have a facilitatory role in the induction of LTP in the PFC. Our findings are similar to those of a previous study that looked at the effects of enhanced DA output using ventral tegmental area (VTA) stimulation on hippo-PFC LTP and reported that VTA stimulation enhanced hippo-PFC LTP when given with weak HPC tetanus (Gurden et al. 1999). The role of LC was further confirmed by inactivation of the LC by local infusion of the local anaesthetic, lidocaine. This reduced hippo-PFC LTP.

The next part of our experiment involved the use of pharmacological agents to modulate the NA system. First, we examined the effects of enhancing NA release on hippo-PFC LTP. To this end we utilized nisoxetine (a selective noradrenergic reuptake inhibitor) and idazoxan (an $\alpha_2$ adrenoceptor antagonist) which are known to enhance NA release in the PFC by different mechanisms. Both drugs significantly enhanced LTP in hippo-PFC pathway of the rat. Nisoxetine increases availability of NA by inhibiting NA removal from the extracellular synaptic spaces by the NA reuptake pump. At 1 mg/kg, nisoxetine shows little non-specific activity (Wong et al. 1982). Idazoxan, on the other hand, increases LC cell firing and release of NA in the PFC through $\alpha_2$ autoreceptor antagonism. Previous studies have shown that NAergic reuptake inhibitors and $\alpha_2$ adrenoceptor antagonists are capable of enhancing NA release in the PFC when administered either by local infusion or systemically (Gesch et al. 1995; Sacchetti et al. 1999). While LTP induced by this protocol in the hippo-PFC pathway is reported to last at least 4 h (Laroche et al. 1995), the observed effects on the amplitude of LTP were evident from the induction of LTP and probably reflect modulation of the induction of early LTP.

Next, we looked at the effects of limiting NA availability on hippo-PFC LTP. The first approach utilized systemic administration of clonidine at 1 mg/kg. Clonidine is an $\alpha_2$ adrenoceptor agonist which mimics the effect of endogenous NA in inhibiting the release of the neurotransmitter (Van Gaalen et al. 1995; Sacchetti et al. 1999). Although it is well established that the $\alpha_2$ adrenoceptor drugs modulate LC firing and NA release in the PFC, they may also have actions at post-synaptic $\alpha_2$ adrenoceptors in the

**Fig. 4.** Effects of noradrenergic drugs on long-term potentiation (LTP) in the hippo-PFC pathway. 
(a) Representative post-synaptic potentials (PSPs) recorded from PFC of saline-, clonidine- and nisoxetine-treated rats before and after two series of hippocampal high-frequency simulation (HFS). The increase in amplitude following HFS was lower in the clonidine group and greater in the nisoxetine group (scale: 0.4 mV, 10 ms). (b) Changes in hippo-PFC post-synaptic response amplitude are plotted over time for saline ( ), n = 5), clonidine ( , n = 5) and nisoxetine ( *, n = 4) treated animals. While clonidine (1 mg/kg i.p.) impaired LTP, nisoxetine (1 mg/kg i.p.) caused an augmentation of LTP. Each point on the graph represents the mean ± S.E.M. of averaged PFC responses to four test stimuli given at 30-s intervals. PSP amplitude is expressed as a percentage of the mean amplitude during 10 min of baseline recording before drug injection. Arrowheads indicate HFS. Drug injection is represented by an arrow. (c) LTP in saline-, clonidine- and nisoxetine-treated groups over different time periods. The columns represent mean ± S.E.M. of the average normalized PSP amplitude in consecutive 20-min periods before and after HFS, respectively ( * $p < 0.05$, ** $p < 0.01$, compared to saline group).
In the second approach, we used a DSP-4 NAergic lesion model. Previous studies have reported the effective use of DSP-4 to destroy large numbers of NAergic fibres that originate in the LC and project to the frontal cortex in rodents (Grzanna et al. 1989). Lesioned animals were studied 1 wk after being given systemic DSP-4. Although the reduction in the amplitude of LTP was not as drastic as that seen with clonidine, LTP was lower in DSP-4-lesioned animals and seemed to decline faster than in controls. When

Figure 5. Effects of cortical NA depletion by DSP-4 on hippo-PFC long-term potentiation (LTP). (a) Representative post-synaptic potentials (PSPs) recorded from PFC of vehicle-treated and DSP-4-treated rats before and after two series of hippocampal high-frequency stimulation (HFS). The increase in amplitude following HFS was lower in the DSP-4-treated group (scale: 0.4 mV, 10 ms). (b) Time-course of LTP in non-lesioned rats (□, n = 5) and rats with DSP-4 lesions (○, n = 7). LTP was reduced in the DSP-4-lesioned rats. Each point on the graph represents the mean ± S.E.M. of averaged PFC responses to four test stimuli given at 30-s intervals. PSP amplitude is expressed as a percentage of the mean amplitude during 10 min of baseline recording before tetanus. Arrowheads indicate HFS. (c) LTP in vehicle-treated and DSP-4-treated groups over different time periods. The columns represent mean ± S.E.M. of the average normalized PSP amplitude in consecutive 20-min periods before and after HFS, respectively (* p < 0.05 compared to vehicle group). (d) Light photomicrograph showing a representative example of Dj/H-immunopositive fibres in the prelimbic area of the medial PFC of non-lesioned rats. Many Dj/H-immunopositive fibres were observed. (e) Light photomicrograph showing a representative example of Dj/H-immunopositive fibres in the prelimbic area of PFC of rats treated with DSP-4. The density of Dj/H-immunopositive fibres was markedly reduced in the DSP-4-lesioned group (scale bar, 50 µm). Inset: Drawings of representative sections showing (boxes) the regions of the prelimbic area of the medial PFC sampled. Drawings are adapted from Paxinos and Watson (1997). PrL, prelimbic cortex (scale bar, 2 mm).
the DBH innervation in the PFC was semi-quantitatively measured, a loss of 84.4% of DBH immunoreactivity was measured in the DSP-4-lesioned animals compared to the control group. It is remarkable that hippo-PFC cortical LTP is still possible with such a large loss of DBH in the PFC terminal regions of the LC projections. Previously, several studies measured NA outflow in LC terminal regions in DSP-4-lesioned animals and found no change, or even an increase, in NA release in the PFC in the presence of cell body degeneration (Hughes & Stanford, 1998; Kask et al. 1997). It is possible that this paradoxical increase in NA outflow allows for development of LTP but is not sufficient to maintain the increase in the post-synaptic response for a prolonged period of time. That neither local infusion of local anaesthetic into the LC nor lesion of noradrenergic projections by DSP-4 had as great an effect on hippo-PFC LTP as clonidine may also suggest that the actions of clonidine involve additional post-synaptic effects.

There are two potential mechanisms by which NA may modulate hippo-PFC LTP. The most direct mechanism is via the interaction of NA with its receptors and the downstream intracellular secondary messenger systems to affect synaptic plasticity in the PFC. cAMP and Ca$^{2+}$ are the key regulators of LTP in the HPC and PFC (Frey et al. 1993; Jay et al. 1998). A recent study demonstrated the ability of NA to increase excitability in PFC pyramidal neurons through enhancement of Ca$^{2+}$ signalling (Barth et al. 2007). Furthermore, the signalling pathways downstream of adrenoceptors involve cAMP or Ca$^{2+}$ (Hein, 2006). It is possible that NA could influence the induction of hippo-PFC LTP through specific changes in the initial level of cAMP and Ca$^{2+}$. By analogy to modulation of LTP in the hippocampal formation, it might be speculated that β adrenoceptors are of particular importance (Gelinas et al. 2008; Huang & Kandel, 1996). A second possible mechanism involves the interaction between NA and DA. It is known that manipulations influencing NA levels can affect DA levels at the same time (Ashby & Tassin, 1996). For example, it has been shown that LC stimulation can modulate activity of midbrain DA (Grenhoff et al. 1993). Devoto et al. (2005) demonstrated that LC

Fig. 6. Enhancement of hippo-PFC long-term potentiation (LTP) by the α₂ adrenoceptor antagonist, idazoxan, in freely moving rats. (a) Representative post-synaptic potentials (PSPs) recorded from PFC of saline- and idazoxan-treated rats before and after hippocampal high-frequency simulation (HFS) twice. The increase in the PSP amplitude following tetanus was greater in the idazoxan group (scale: 0.4 mV, 10 ms). (b) Changes in hippo-PFC post-synaptic response amplitude are plotted over time for saline (□, n = 4) and idazoxan (●, n = 4) treated animals. Idazoxan (4 mg/kg i.p.) facilitated LTP. Each point on the graph represents the mean ± S.E.M. of averaged PFC responses to four test stimuli given at 30-s intervals. PSP amplitude is expressed as a percentage of the mean amplitude during 10 min of baseline recording before drug injection. Arrowheads indicate HFS. Drug injection is represented by an arrow. (c) LTP in saline- and idazoxan-treated groups over different time periods. The columns represent mean ± S.E.M. of the average normalized PSP amplitude in consecutive 30-min periods before and after HFS, respectively (* p < 0.05 compared to saline group).
stimulation can elicit a co-release of DA and NA in the PFC. It is also well-known that $\alpha_2$ adrenoceptor agonists and antagonists can alter extracellular levels of both NA and DA in the PFC (Gresch et al. 1995). In the case of nisoxetine, it is postulated that it increases the extracellular concentration of DA in the PFC by blocking the NA transporters that normally clear both NA and DA from synaptic spaces (Carboni et al. 1990; Yamamoto & Novotney, 1998). Since Jay and colleagues have shown that DA can modify hippo-PFC LTP (Jay, 2003), it is possible that the enhancement of hippo-PFC LTP could have resulted from changes in the NA level alone, from changes in DA release secondary to alterations in NA levels or from a combination of effects of both neurotransmitters. The last option seems to be the most reasonable since the behavioural performance dependent on the hippocampal pathway appears to be modulated by both $\beta$ adrenoceptors (Tronel et al. 2004) and D$_1$ receptors (Seamans et al. 1998).

In conclusion, we found that hippo-PFC synaptic plasticity is modulated by NA availability. In addition, enhancement of LTP in the hippo-PFC pathway following idazoxan in the freely moving rat further indicated that the effect of NA on hippo-PFC could be relevant to behaviour. Many psychiatric medications, especially antipsychotic drugs and antidepressants, affect the NAergic system and could possibly exert their effects on cognition through this pathway. Elucidating the mechanisms that govern synaptic plasticity in pathways related to cognition will help us to verify the specific pharmacological targets by which new drugs can be formulated to alleviate the cognitive symptoms of these psychiatric disorders.

**Note**

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

**Acknowledgements**

This work was supported by the Biomedical Research Council, Agency for Science Technology and Research, Singapore (grant numbers 03/1/21/18/269 and 06/1/21/19/458) and by an award to both G.S. Dawe and T.M. Jay from the MERLION programme for joint French–Singaporean research. We thank A/Professor Sanjay Khanna for his advice on electrophysiology. We are grateful to Mdm Rajini Nagarajah, Ms Siew Ping Han and Mr Woon Fei Ho for their excellent administrative support and technical assistance.

**Statement of Interest**

None.

**References**


Floresco SB, Seamans JK, Phillips AG (1997). Selective roles for hippocampal, prefrontal cortical, and ventral striatal...
circuitst in radial-arm maze tasks with or without a delay. *Journal of Neuroscience* 17, 1880–1890.


Lyons WE, Fritschy JM, Grzanna R (1989). The noradrenergic neurotoxin DSP-4 eliminates the coeruleospinal projection but spares projections of the A5 and A7 groups to the ventral horn of the spinal cord. *Journal of Neuroscience* 9, 1481–1489.


Ohashi S, Matsumoto M, Togashi H, Ueno K, et al. (2003). The serotonergic modulation of synaptic plasticity in...


