JNeurosci
THE JOURNAL OF NEUROSCIENCE

Research Articles | Behavioral/Cognitive

Functional and regional specificity of noradrenergic signalling for encoding and retrieval of associative recognition memory in the rat

https://doi.org/10.1523/JNEUROSCI.2408-24.2025

Received: 20 December 2024
Revised: 24 April 2025
Accepted: 27 April 2025

Functional and regional specificity of noradrenergic signalling for encoding and retrieval of associative recognition memory in the rat

Abbreviated title: Noradrenaline and associative recognition memory

Stephanie Tran[1], Gareth R.I. Barker[1], Mathias L. Mathiasen[2] John P. Aggleton[2] and E. Clea Warburton[1]

1. School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol BS8 1TD, U.K
2. School of Psychology, Cardiff University, Tower Building, 70 Park Place, Cardiff, CF10 3AT

Corresponding author: EC Warburton email e.c.warburton@bristol.ac.uk

Number of Figures	3
Number of Tables	7
Number of words for Abstract	250
Number of words for Introduction	493
Number of words for Discussion	1500

Conflict of interest statement: The authors declare no conflicts of interest

Acknowledgements: This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC) studentship to S.T. (Grant/Award Number: BB/M009122/1) and a Wellcome Trust Investigator Award to E.C.W. (Grant/Award Number: 206401/Z/17/Z)

ABSTRACT

Recognition of a familiar object in a novel location requires retrieval of the former object-place association and encoding of novel information. Such object-in-place memory (OiP) recruits a neural network including the hippocampus (HPC), medial prefrontal cortex (mPFC) and nucleus reuniens of the thalamus (NRe), however, the underlying cellular mechanisms are not understood. Locus coeruleus (LC) noradrenergic neurons signal novelty, thus here we focussed on the contribution of LC-forebrain projections, and noradrenaline (NA) receptor subtypes to OiP encoding compared to retrieval, using an arena-based OiP task in male rats. The NRe was found to receive a catecholaminergic input from LC, with the strongest innervation directed to rostral NRe. Interestingly optogenetic inactivation of the LC→NRe pathway impaired OiP retrieval but was without effect on encoding while inactivation of the LC→HPC selectively impaired encoding. Consistent with this double dissociation, pharmacological blockade of NRe α1-adrenoreceptors selectively impaired memory retrieval, while blockade of HPC β-adrenoreceptors impaired encoding. Finally, pharmacological attenuation of noradrenergic signalling in the NRe and HPC through the infusion of the α2-adrenergic receptor agonist UK14,304 impaired retrieval and encoding respectively. Surprisingly, antagonism or agonism of adrenoreceptor subtypes in the mPFC had no effect on memory performance. Together these results reveal the importance of NA within the HPC and NRe for OiP whereby selectivity of function is achieved via spatially distinct LC output projections and NA receptor subtypes consistent with a modular view of NA function. These results are also important in demonstrating the distinct neuronal mechanisms by which encoding, and retrieval are achieved.

Significance Statement

Noradrenaline projections from the locus coeruleus (LC) have been recognised as providing a novelty signal to the forebrain yet whether this signal is important in mediating different stages of memory is poorly understood. Our results demonstrate that associative recognition memory retrieval is selectively mediated by a direct projection from the LC to the nucleus reuniens of the thalamus (NRe) and by activation of NRe α1 and α2 -adrenoreceptors. Conversely encoding is selectively mediated by LC input to hippocampus and by hippocampal β and α2 -adrenoreceptors. These findings reveal functional and regional specificity of noradrenergic modulation of memory processing in the context of memory circuitry and thus enables the definition of clearer targets for disease-modifying therapies for patients with memory deficits.

INTRODUCTION

Remembering a stimulus such as an object and the location in which it was last encountered is a crucial memory process. Such memories can be formed rapidly in a 'one-shot' encoding of an object-in-place association (OiP), while retrieval of this association enables the rapid detection of a change in our environment. We have previously identified a hippocampal (HPC) - medial prefrontal cortex (mPFC) - nucleus reuniens of the thalamus (NRe) network, in which specific neural pathways differentially mediate encoding and retrieval of OiP (Barker et al., 2021), yet the underlying cellular mechanisms by which these processes are mediated are poorly understood.

Stimulus novelty is a key factor in driving memory encoding (Dunsmoor et al., 2022) and exploration of novel objects or environments produces significant increases in neuronal firing within the locus coeruleus (LC) (Sara et al., 1994; Vankov et al., 1995), the origin of forebrain noradrenaline (NA) afferents, suggesting that the LC provides a key signal which drives memory formation. Consistent with this hypothesis, behavioural studies show that activity in the LC→HPC and LC→mPFC projections is critical for spatial memory encoding and contextual fear learning while blockade of hippocampal α1-adrenergic or β-adrenergic receptors impairs spatial memory learning (Lemon et al., 2009; Fan et al., 2022; Torkaman-Boutorabi et al., 2014; Tsetsenis et al., 2022). Although the role for NA in encoding is clear, for retrieval it is less so. For example, in one study, retrieval of a contextual fear memory was found to require a decrease in NA release in the HPC (Wilson et al., 2024), in contrast increased NA in the basolateral amygdala enhanced retrieval (Fukabori et al., 2020). Thus, evidence indicates that NA neurotransmission is involved in both memory encoding and retrieval, yet its role is clearly complex and may differ depending on the brain region and/or task under investigation.

As stated, OiP memory depends on a HPC-NRe-mPFC network (Barker et al., 2021). While the LC-NA projections to HPC and mPFC are numerous and well described (Loughlin et al., 1986; Smith and Greene, 2012), the NRe has been reported to receive only limited input (Lindvall et al., 1974; Swanson and Hartman, 1975; McKenna and Vertes, 2004) despite appearing to have dense adrenoreceptor expression (Sargent-Jones et al., 1985; Boyajian et al.,1987; Palacios and Kuhar, 1982). Thus, it is likely that the density of NA innervation to the NRe is more extensive than previously described and if so, may have an important role in the neuromodulation of encoding or retrieval of associative recognition memory.

To examine the relationship between forebrain NA and the encoding and retrieval of associative recognition memory we took a circuit analysis approach using; i. anatomical tracing techniques to map the extent of catecholaminergic projections from the LC to NRe, ii. combined optogenetic and recognition memory testing to assess the importance of LC inputs to the HPC and NRe on encoding and retrieval, iii. selective pharmacological manipulations of adrenergic receptor subtypes to establish their relative contribution to OiP encoding and retrieval.

MATERIALS AND METHODS

Animals

For the anatomical studies, 8 male Lister Hooded rats (Envigo, UK) weighing 297-307g at the start of experimentation were used. Rats were group housed (2-4 per cage) kept on 12-hour light/dark cycle (light phase, 06:00 to 18:00). For the behavioural studies, 48 male Lister Hooded rats (Harlan Laboratories, UK) weighing 300-400g at the start of experimentation were used. For the optogenetic

experiments, animals were split into 2 groups: those that received the control virus, AAV5-CaMKII-EYFP (YFP, n = 12), and those that received the virus that expresses the inhibitory opsin, AAV5-CaMKII-eArch3.0-EYFP (Arch, n = 12). For the pharmacology experiments, animals were split into 2 groups: bilateral cannula implanted into the NRe (n=12), and bilateral cannula implanted into the HPC and mPFC (a total of 4 cannula per animal) (n=12). Rats were group housed (2-4 per cage) and kept on 12- hour light/dark cycle (light phase, 18:00 to 06:00). All animals had *ad libitum* access to water and standard chow. All animal procedures were conducted in compliance with the Animals (Scientific Procedures) Act, (1986).

Surgical procedures

General surgical procedures

Animals were anaesthetised using isoflurane (induction 4%, maintenance 2%). The scalp of the animals was shaved before they were positioned in a stereotaxic frame, the incisor bar was adjusted to achieve a flat skull (Kopf Instruments, USA). Before the start of surgery animals received eye drops (0.1% sodium hyaluronate; Hycosan, UK) and topical application of both lidocaine (5% m/m; TEVA; UK) and chlorhexidine on the scalp. Following surgery, the skin was sutured and antibiotic wound powder (2% w/w; Battle, UK) was applied. Immediately post-surgery animals received eye drops (0.1% sodium hyaluronate; Hycosan, UK), subcutaneous injection of 5ml glucose saline (sodium chloride 0.9% w/v with glucose 5% w/v), intramuscular injection of 0.05ml vetergesic (0.3 mg/ml buprenorphine; Ceva Animal Health, UK) and intramuscular injection of 0.1ml Clamoxyl (150mg/ml; Zoetis, UK).

Injection of anatomical tracers

Each animal received a unilateral injection of an anatomical tracer into the NRe. All tracer injections were given at a 6° angle from the mediolateral plane. The stereotaxic co-ordinates were derived from the rat brain atlas of Paxinos and Watson (2007). As the NRe lies directly ventral to the sagittal sinus, mediolateral co-ordinates used were aimed to target as close to the side of the sagittal sinus as possible. Table 1 shows a list of cases including details of the co-ordinates used, anatomical tracer used and the main site of tracer deposit. For pressure injections, Fast Blue (FB) or Cholera Toxin B Subunit (CTB) were mechanically injected via a 1µl Hamilton syringe (Hamilton, Bonaduz, Switzerland), 55nl was injected per site at a rate of 20nl/min. The syringe was left in situ for 3 min prior to injection and 10 min after injection to minimize leakage of tracer. For iontophoretic injections, CTB or FluoroGold (FG) were injected using a glass micropipette (tip diameter 15-20µm). A positive pulsed current (2µA for 6 min followed by 6µA for 6 min and finally 7µA for 6 min) was applied using Digital Midgard Precision Current Source iontophoretic pump (Stoelting Co, Wood Dale, USA) on a cycle of 6 seconds on/ 6 seconds off. After the injection period, the glass micropipette was left *in situ* for 3 min to minimise leakage of tracer. During withdrawal of the micropipette a negative current was applied. All animals were allowed to recover for 7 days before being sacrificed for subsequent histological processing.

Viral injections and implantation of optical fibres

Animals received a bilateral injection of AAV5-CaMKII-eArchT3.0-EYFP (Arch group) or AAV5-CaMKII-EYFP (YFP group) into the LC. To target the LC the following co-ordinates were used: AP -9.6mm, ML ±1.4mm, DV -7.4mm. Each animal received 2 injections (1 in each hemisphere) of virus through a 5µl Hamilton syringe. Each virus was injected at a rate of 0.2µl/min using a Micro4 controller infusion pump (World Precision Instruments, USA), attached to the arm of the stereotaxic frame. The needle was left in situ for a further 10 min before being withdrawn. Following injection of the virus, animals were immediately implanted with bilateral optical fibres to target both the NRe and HPC.

Therefore, for a given animal 4 optical fibres were implanted (2 aimed at the NRe and 2 aimed at the HPC). To implant optical fibres, burr holes were drilled into the skull to allow implantation of optical fibre (core = 200µm, numerical aperture = 0.22, (MFC 200/240-0.22 SM3 C45 Mono Fiberoptic Cannula); Doric Lenses, Quebec, Canada). Four stainless steel screws (Plastics One, Bilaney, UK) and dental cement were used to anchor the optical fibres. To target the NRe animals were implanted with bilateral optical fibre (length: 7mm) using the following co-ordinates: AP -1.8mm, ML ±2mm, DV -6.6mm. All optical fibres were implanted 15° from the ML plane. To target the HPC animals were implanted with bilateral optical fibre (length: 5.5mm) using the following coordinates: AP -5.4mm, ML ±2.7mm, DV -2.8mm. All optical fibres were implanted 25° from the AP plane.

Cannula implantation

Burr holes were drilled into the skull to allow implantation of stainless-steel guide cannula (26 gauge; Plastics One, Bilaney, UK). Four stainless steel screws (Plastics One, Bilaney, UK) and dental cement were used to anchor the cannula. To target the NRe, animals were implanted with bilateral cannula using the following coordinates: anterior-posterior (AP) -1.8mm and -2.4mm; mediolateral (ML) ±1.7mm, dorsoventral (DV) -6.4mm. All cannula were implanted 15° from the ML plane. To target the HPC or mPFC, animals were implanted with bilateral cannula to target both brain regions. Therefore, for a given animal 4 infusion cannula were implanted (2 aimed at the HPC and 2 aimed at the mPFC). To target the HPC, the co-ordinates were: AP -4.3mm, ML ±2.5mm, DV -2.8mm (dura). To target the mPFC, the co-ordinates were: AP +3.2mm; ML ±0.75mm, DV -3.5mm. To prevent contamination/cannula blockages, dummy cannula were inserted into the guide cannula. Animals were singly housed for seven days post-surgery and given two weeks to recover before behavioural testing commenced.

Behavioural procedures

Behavioural apparatus and habituation

Behavioural testing was conducted in a wooden open-topped (90cm x 100 cm x 50cm) arena with a sawdust covered floor. One wall of the arena was black, and three other walls grey in colour on one side and surrounded by a black cloth on the north and south side hung from a height of 1.5m. The room was lit with two floor lamps situated at either side of the arena. A webcam was located above the arena to record behaviour. Objects were constructed from Duplo blocks (Lego, Denmark) and varied in size (ranging from 16x16x8cm to 20x20x25cm), colour and shape. Objects were placed 10cm from the edges of the arena and cleaned with 100% ethanol during the delay period between sample and test and between animals to remove olfactory cues. All animals were handled extensively prior to habituation and then habituated to the behavioural testing setup for 4 days before memory testing.

Drugs and infusion procedure for cannulation experiments

The following drugs were used: the α2 adrenergic agonist UK 14,304 (2466, Tocris, UK); the α2 antagonist RS 79948 (0987, Tocris, UK); the α1 antagonist prazosin (0623, Tocris, UK); and the β adrenergic antagonist propranolol (0834, Tocris, UK). UK 14,304, propranolol and RS 79949 were dissolved in 0.9% sterile saline solution and infused at the following concentrations: UK 14,304 (10μM); propranolol (10μM); and RS 79948 (1μM). Prazosin was initially dissolved in 100% dimethyl sulfoxide (DMSO), the stock solution was subsequently diluted with 0.9% sterile saline solution, yielding an infusion concentration of 1μM prazosin in 0.1% DMSO. For the NRe experiments, vehicle

control animals received either 0.9% sterile saline solution (UK 14,304 and RS 79948 experiment) or 0.9% sterile saline solution with 0.1% DMSO (prazosin and propranolol experiment). For the HPC-mPFC experiments, vehicle control animals received 0.9% sterile saline solution. Drug doses used were based on published IC50 values (Atlas et al., 1974; Bylund and Snyder, 1976; Greengrass and Bremmer, 1979; Lefkowitz et al., 1976; U'Prichard et al., 1978; van Meel et al., 1981). Drugs were infused via 33-gauge cannula (Plastic Ones, Bilaney, UK) attached to a 25µl Hamilton syringe by polyethylene tubing. Rate of infusion was controlled using an infusion pump (Harvard, UK). For the NRe, animals were infused with 0.3µl of drug or saline per hemisphere at a rate of 0.3µl/min. For the HPC, animals were infused with 0.5µl of drug or saline per hemisphere at a rate of 0.25µl/min. For mPFC infusions, animals were infused with 1µl of drug or saline per hemisphere at a rate of 0.5µl/min. Following infusion, cannulae were left in place for 5 min. Infusions were given 15 min before the sample phase to test the effects on encoding or 15 min before the test phase to assess the effects on retrieval.

Stimulation protocol for optogenetic experiments

Laser light for optical stimulation was generated using a diode laser (Omicron LuxX® 515-100 laser (515nm), Photonlines, UK). The laser was attached to a fibre optic rotary joint with beam splitter (FRJ 1X2i FC-2FC, Doric Lenses, Quebec, Canada) via a fibre-optic patch cord (core = 200µm, numerical aperture = 0.22, FG200LEA, ThorLabs, Newton, NJ, USA). Two fibre-optic patch cords (core = 200µm, numerical aperture = 0.22, FC-CM3, Doric Lenses, Quebec, Canada) were attached to the rotary joint at one end while the other end was used to connect to the optical implant on the animal's head. The power output of the laser was adjusted so that 10mW was measured at the tip of each optical fibre. Optical stimulation was either given throughout the length of the sample phase to test the effects on encoding or throughout the length of the test phase to test the effects on retrieval. Laser

stimulation was delivered at a frequency of 30 Hz and a duration of 10ms pulses (50% duty cycle) using a custom protocol on WinLTP (2.20 M/X-Series, WinLTP Ltd.). Stimulation parameters were chosen based on a previous in vitro electrophysiological study conducted in acute brain slices demonstrating that laser stimulation using the abovementioned parameters resulted in a robust decrease in resting membrane potential (Banks et al., 2021).

Spontaneous object recognition tasks

The object-in-place task (OiP) comprised a sample and test phase, separated by a 3-hour delay (Figure 2E). In the sample phase (5 min) each animal was placed in the arena which contained four different objects. Each animal was then allowed to explore the objects before being removed from the arena and placed back into the home cage for the delay. For the test phase (3 min), animals were placed back in the arena which contained the same four objects, but two objects had exchanged positions. Successful OiP memory is demonstrated by the animal preferentially exploring the two moved objects (the novel configuration) compared to the two objects in the same position (familiar configuration).

The novel object recognition task (NOR) comprised a sample and test phase with a 3-hour delay (Figure 2I). In the sample phase (5 min) the animal explored four different objects before being removed from the arena and placed in the home cage for the delay. In the test phase, two objects from the sample phase, were replaced with novel objects. Intact NOR is demonstrated by greater exploration of the novel over the familiar objects.

The object location memory task (OL) comprised a sample and test phase with a 3-hour delay (Figure 2J). For the sample phase (4 min) each animal was placed in the arena which contained two identical

objects which they were allowed to explore before being removed from the arena for the delay. Following the delay, the animals were placed back in the arena where the location of one object was changed. Successful OL memory is demonstrated by greater exploration of the familiar object in the new location over the familiar object in the familiar location.

The object-in-place task with two test phases employed similar methods to the OiP task as described above, but consisted of two separate test phases (Figure 2G). At test phase 1, two objects, either those on the left or right side, exchanged positions, and the animals were given 5 min to explore. At test phase 2, two objects either both on the left or right side, exchanged positions, and the animals were given 3 min to explore. If during test phase 1, objects to the left exchanged positions, then during test phase 2, objects to the right exchanged positions and vice versa. If an animal demonstrates successful OiP memory it should preferentially explore the two objects which have exchanged positions (the novel configuration) over the two objects which have remained in the same position (familiar configuration). Thus, in Figure 2G, at test phase 1, animals with intact memory will preferentially explore the objects on the right-hand side of the arena (i.e. the moved objects relative to their position in the sample phase) and at test phase 2, animals will preferentially explore the objects on the left-hand side of the arena (i.e. objects which have been moved relative to their positions in test phase 1).

Behavioural scoring

Total object exploration in the sample and test phases was measured using a custom software with the experimenter blind to the experimental condition of the animal. In all tasks the positioning and/or identity of the objects in the sample and test phases in each task was counterbalanced between the animals. Exploration of an object was measured in seconds and defined as the animal's nose directed towards the object and less than 2cm from the object while actively sniffing. Sitting on top of the object

or using the object for supported rearing was not scored as exploratory behaviour. To measure an animal's ability to discriminate between the novel configuration/object compared to the familiar configuration/object, a discrimination ratio was calculated as follows:

$$\text{Discrimination ratio} = \frac{\text{(exploration of novel (sec) – exploration of familiar (sec))}}{\text{total exploration time (sec)}}$$

A value of zero indicates no preference for the novel or familiar configuration/object. A positive discrimination ratio value indicates a preference for the novel configuration/ object/, while a negative value indicates preference for the familiar object/configuration

Histology

Tissue fixation

On completion of experiments animals received an intraperitoneal injection of sodium pentobarbital (Euthatal, Merial, Harlow, UK). Animals were transcardially perfused with 0.1M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.1M PBS (anatomical tracing, or viral injections/ optical fibre implantation animals) or 4% formal saline (cannulated animals). Brains were removed and post-fixed with PFA for a minimum of 4 hours or with formal saline for a minimum of 1 week before being transferred to 25% sucrose in 0.1M PBS for 24 hours.

Tissue preparation

Following the tissue fixation procedures outlined above, brains were sectioned using a cryostat (Leica CM3050S, Milton Keynes, UK) into 40µm coronal sections. For anatomical tracing and viral

injection/optical fibre implantation animals, four series were taken. The first tissue series was directly mounted onto gelatin-subbed slides for cresyl violet staining. The second tissue series was subject to immunohistochemical processing. For cannulated animals, sections were directly mounted onto gelatin-subbed slides and air dried before staining with cresyl violet. A Leica DM6 B microscope mounted with a Hamamatsu C13440 digital camera was used to image the samples.

Immunohistochemical procedure

Immunohistochemical staining was performed on free-floating sections. Sections were washed with 0.1M PBS (3 x 10 min). Sections were incubated in blocking solution (5% animal serum, 2.5% bovine serum albumin, 0.2% Triton x-100 in 0.1M PBS (PBST)) for 1 hour before incubation with primary antibodies diluted in blocking solution overnight at room temperature. Sections were then washed in 0.1M PBST (4 x 10 min) before incubation in secondary antibodies diluted in blocking buffer for 2 hours at room temperature. Sections were given a final wash with PBS (4 x 10 min) and mounted on gelatin-subbed slides and coverslipped with Fluoromount (Sigma-Aldrich, F4680, St. Louis, MO, USA). The following primary antibodies were used in this study: rabbit anti-TH (1:1000, AB152, Chemicon), chicken anti-TH (1:1000, AB76442, Abcam), chicken anti-GFP (1:1000, GFP-1020, Aves Labs), and rabbit anti-CTB (1:3000, C30620, Sigma).

Anatomical nomenclature

Anatomical boundaries and nomenclature follow the rat brain atlas of Paxinos and Watson (2007), except for terminology regarding noradrenaline-positive neurons which follows the well described nomenclature (Fuxe, 1964; Hokfelt, 1984). To determine the origin of noradrenergic input to the NRe, only noradrenergic cell groups which have previously described projections to the NRe were

examined for double-labelled neurons (i.e., those that demonstrate co-staining of both the retrograde tracer and TH antibody) (McKenna and Vertes, 2004).

Cell counts and quantification

For cell counts, the region of interest was determined by the presence of TH-positive cells. All TH-positive cells, retrogradely transported cells and double-labelled cells within the region of interest were counted for each animal. Olympus cellSens Dimension Desktop Software was used to perform manual cell counts. For cell counts, the region of interest was determined by the presence of TH-positive cells. All TH-positive cells, retrograde tracer-positive cells, and double-labelled cells within the region of interest were counted for each animal. Note the counts were not stereological so should give relative not absolute numbers.

Experimental Design and Statistical Analysis

The cannulation experiments were run with a cross-over design, thus for a given experiment, each animal received both drug and saline infusions. For the HPC-mPFC implanted animals, saline infusion into the HPC or mPFC was counterbalanced between infusion timing, e.g., for a given drug, if an animal received a pre-sample infusion of saline into the HPC, for the pre-test infusion, the same animal would receive saline infusion into the mPFC or vice versa. The optogenetic experiments were run with a cross-over design with each animal tested with both optical stimulation on and off conditions.

In all behavioural experiments, statistical analyses were performed to compare discrimination ratios, sample phase exploration times and test phase exploration times between conditions. In addition, in all experiments to determine whether the discrimination ratio for each condition was significantly

different from chance (a discrimination ratio of zero), one-sample t-tests were conducted. Alpha was set at 0.05 for all analysis. IBM SPSS Statistics 25 software (IBM, USA) was used to perform all statistical analysis. Graphs were created using R 3.6.1 (R Core Team, Austria). Data are presented as mean ± standard error of mean (SEM).

RESULTS

Catecholaminergic innervation of NRe

To visualise the distribution of catecholaminergic innervation to the NRe, an antibody against tyrosine hydroxylase (TH) was used. As depicted in Figure 1A-C, the entire rostro-caudal axis of the NRe contained TH-immunopositive fibres (TH+) that were fine and spindly in nature. Interestingly the distribution of TH+ fibres in the NRe was non-uniform. At the rostral-most level (Figure 1A), moderate levels of labelled fibres were observed, whereas fewer labelled fibres were observed in the intermediate to caudal levels (Figure 1B; 1C). There was no apparent variation in the density of TH+ fibres in the medio-lateral plane.

To examine whether the LC provided a catecholaminergic input to the NRe, we employed retrograde labelling using cholera toxin subunit B (CTB), Fast Blue (FB) or Fluorogold (FG) combined with TH immunohistochemistry. In all cases analysed (see Figure 1D for an overview of cases), double-labelled neurons, i.e., neurons immunopositive for both CTB/FB/FG and TH, were observed in the A6 - LC (Figure 1F) but were rarely observed from other noradrenergic cell groups analysed (A7 pontine reticular formation (data not shown)). In the LC 63.0% of cells were retrograde+/TH+ and in those cases where the position of the NRe injection was more rostral (cases #1, #2, #3, #4, #7), there was a greater proportion of double-labelled cells in the LC, compared to the more caudal injections (cases

#5, #6) (Figure 1D; 1G). These findings suggest that the LC may provide a stronger catecholaminergic input to the rostral compared to caudal NRe, although further studies are needed to confirm this.

Dissociation of the role of LC projections to NRe and HPC on OiP encoding and retrieval

In view of the observed strong projections from LC to NRe, and the previously reported evidence that the LC projection to the HPC is crucial for some forms of memory (Kempadoo et al., 2016; McNamara and Dupret, 2017; Takeuchi et al., 2016; Wagatsuma et al., 2017), we next used specific optogenetic pathway inhibition to assess the functional roles of the LC→NRe and LC→HPC projections on encoding and retrieval of associative recognition memory. Animals received bilateral injection of Arch or YFP into the LC, followed by bilateral implantation of optrodes into the NRe and HPC. Animals were allowed to recover for 6 weeks before behavioural testing commenced (Figure 2A). Following behavioural testing, immunohistochemistry confirmed that viral expression was observed in the LC (Figure 2B) with axonal transport of virus, as well as optrode placement targeting the NRe (Figure 2C) and HPC (Figure 2D).

Figure 2E shows the discrimination ratios when light was delivered during the sample phase into the NRe or HPC of the Arch or YFP groups compared to a 'light off' condition. We found that light delivery in the Arch-HPC, but not the Arch-NRe group, significantly impaired OiP performance. These results are supported by a significant interaction between stimulation and virus in the ANOVA ($F_{(2,44)} = 4.06$, $p = 0.024$) and Bonferroni-corrected paired t-test: Arch-Off vs Arch-NRe: ($p = 1.00$ n.s.); Arch-Off vs Arch-HPC: ($p = 0.003$); Arch-NRe vs Arch-HPC: ($p = 0.013$)). We next examined the effects of light delivery during the test phase (Figure 2F) and found impairment in the Arch-NRe but not the Arch-

HPC group (stimulation by virus interaction $F_{(2,44)} = 5.64$, $p = 0.007$) Bonferroni-corrected paired *t*-test (Arch-Off vs NRe: ($p = 0.029$); Arch-Off vs Arch-HPC: ($p = 1.00$); Arch-NRe vs Arch-HPC: ($p = 0.024$)).

The double dissociation, i.e. that LC→HPC is required for OiP encoding, while LC→NRe is selectively required for OiP retrieval suggests a separation of the function of the two pathways. However, encoding of new information and retrieval of old information co-occur during ongoing behaviour, which raises the question of whether it is possible to block one process whilst leaving the other intact. To address this question, we adapted the OiP task, to include two test phases with light stimulation delivered only during test phase 1 (Figure 2G, top). If encoding and retrieval are truly mediated by separate neural projections, we hypothesised that LC input to NRe will be required for retrieval of the object-place configurations encoded during the sample phase, as assessed during the first test phase, but not for the encoding the novel object-place configurations encountered during that test phase (i.e. test phase 1) which will be dependent on the LC input to HPC. The discrimination ratios following light delivery into the Arch-HPC and Arch-NRe for test phase 1 and test phase 2 are shown in Figure 2G. As expected performance in test phase 1 was impaired following light delivery in the Arch-NRe, but not the Arch-HPC condition (ANOVA stimulation x virus ($F_{(2,44)} = 6.03$, $p = 0.005$). Bonferroni corrected paired *t*-test: (Off versus NRe: ($p = 0.002$)), (Off versus HPC: ($p = 1.00$), (NRe versus HPC: ($p = 0.004$)). In contrast performance in test phase 2 was impaired following light delivery in the Arch-HPC, but not the Arch-NRe condition (ANOVA stimulation x virus: $F_{(2,44)} = 3.48$, $p = 0.040$). Bonferroni corrected post-hoc analysis (Off versus Arch-NRe: ($p = 1.00$), (Off versus Arch-HPC: ($p = 0.043$), (Arch-NRe versus Arch-HPC: ($p = 0.020$)).

Finally, we examined the effects of optogenetic inhibition of the LC→HPC and LC→NRe pathways during the sample or test phases of the NOR or OL tasks (Figure 2H; 2I; 2J; 2K) and no impairments in performance were observed. Further analysis revealed that overall exploration levels in all tasks

were not affected (Table 2). In addition, all observations were confirmed by comparing performance against chance i.e. discrimination of zero (Table 3).

α1, α2 and β -adrenergic receptors play a regionally specific role in object-in-place memory

Given the differential roles of LC innervation of the HPC and NRe on OiP encoding and retrieval we next examined the role of specific adrenergic receptor subtypes in the NRe and HPC. In these experiments we also included a group in which infusions were made into the mPFC for a number of reasons; i. neuronal activity in the mPFC is key for associative recognition memory encoding and retrieval (Barker et al., 2007; Barker and Warburton, 2011; Benn et al., 2016); ii. the mPFC receives a significant NA input (Agster et al., 2013; Waterhouse and Chandler, 2012; Cerpa et al., 2019); iii. we have previously found a selective role for D1/D5 receptors in the mPFC for OiP memory encoding, but not retrieval; iv. the role of NA receptors in the mPFC have not yet been examined.

Two groups of animals received surgery to bilaterally implant chronically indwelling cannulae aimed, in one group at the NRe only (Figure 3A), or in the second group at both the HPC and mPFC (Figure 3B). The cannulae allowed local administration of selective receptor antagonists prazosin (α1-adrenergic antagonist), propranolol (β-adrenergic antagonist), UK 14,304 (α2-adrenergic receptor agonist) or RS79948 (α2-adrenergic receptor antagonist), either before the sample phase, to investigate effects on encoding, or before the test phase to investigate effects on retrieval (Figure 3C).

Intra-NRe administration of either prazosin or propranolol before the sample phase had no effect on OiP performance (Figure 3D), however, when the infusions were delivered prior to the test phase, prazosin, but not propranolol significantly impaired performance, as confirmed by significant drug x infusion timing interaction ($F_{(2,36)}$ = 4.09, p = 0.025). Bonferroni corrected post-hoc t-test: pre-test vehicle vs prazosin (p =0.006); prazosin vs propranolol (p = 0.011); vehicle vs propranolol (p = 1.00 n.s.).

Local infusion of prazosin into either the HPC or mPFC, pre-sample or pre-test had no effect (Figure 3E) (region x infusion timing ($F_{(2,44)}$ = 0.222, ns). In contrast, pre-sample intra-HPC administration of propranolol, produced a significant memory disruption while pre-sample intra-mPFC infusions had no effect (Figure 3F) (region x infusion timing ($F_{(2, 40)}$ = 3.73, p = 0.033; Bonferroni corrected post-hoc t-test pre-sample infusion timepoint: (Vehicle vs HPC (p = 0.017); Vehicle vs mPFC (p = 1.00); HPC vs mPFC (p = 0.006)). Together these results show that α1-adrenergic receptors in the NRe are critical for retrieval, while β-adrenergic receptors in HPC are critical for OiP encoding.

In the final series of experiments, we investigated the effects of inhibiting or stimulating NA release, by local infusion of the α2-adrenergic receptor agonist (UK 14,304) or antagonist (RS 79948). As α2-adrenergic receptors exist, although not exclusively, as autoreceptors, located presynaptically on the terminals of noradrenergic neurons (Starke, 2001; Langer, 1974; Milner et al., 1998). Previous microdialysis studies have shown that UK 14,304 infusions cause a robust decrease in NA levels (Dalley and Stanford, 1995; Ferry et al., 2015; van Veldhuizen et al., 1993) while infusion of RS 79948 results in a robust increase in NA (Fernández-Pastor and Meana, 2002; Horrillo et al., 2019). Here we found that intra-NRe infusion of UK 14,304 before the test, but not before the sample phase significantly impaired discrimination (Figure 3G) (drug x infusion timing ($F_{(1,18)}$ = 6.29, p = 0.022)); Bonferroni corrected post-hoc t-test pre-sample (Vehicle vs UK 14,304 ($t_{(9)}$ = -0.462, n.s.): pre-test

445 (Vehicle vs UK 14,304 ($t_{(9)}$ = 3.62, p = 0.006). In contrast intra-HPC infusion of UK 14,304 prior to the
446 sample, but not test, impaired discrimination while infusions into the mPFC had no effect (Figure 3H)
447 (region x infusion timing ($F_{(2,44)}$ = 2.67, p = 0.080 n.s.). Comparisons against chance showed that pre-
448 sample infusion into the HPC significantly impaired discrimination ($t_{(11)}$ = 1.45 p = .176) while all other
449 groups significantly discriminated vehicle (pre-sample ($t_{(11)}$ = 4.19, p =0 .002); pre-test ($t_{(11)}$ = 3.98, p
450 = 0.002)); mPFC (pre-sample ($t_{(11)}$ = 5.69, p < 0.001); pre-test ($t_{(11)}$ = 3.20, p = 0.008))., HPC (pre-test
451 ($t_{(11)}$ = 5.41, p < .001). When we tested the effect of RS 79948 into the NRe, HPC or mPFC we found
452 no effects on memory performance irrespective of brain region or timing of infusion (Figure 3I; 3J)
453 confirmed by ANOVA (NRe: drug x infusion timing interaction ($F_{(1, 16)}$ = 0.001, p = .978)) (HPC vs
454 mPFC: region x infusion timing ($F_{(2,44)}$ = 0.003, p = 0.997)).

455

456 Analysis of total object exploration during the sample and test phases indicated overall exploration
457 levels in all tasks were not affected. While some analyses revealed significant main effect of infusion
458 timing, further analysis revealed that this effect was importantly independent of infusion region and
459 due to differences observed in exploration times when either pre-sample or pre-test infusions were
460 given. (Table 4; Table 6). In addition, analysis comparing performance against chance confirmed
461 these observations (Table 5; Table 7). Together, these results support our conclusions that that
462 successful OiP encoding and retrieval requires release of NA in the HPC, and NRe respectively.

463

464 **DISCUSSION**

465 This study contains several important new findings. We showed, for the first time, that the entire rostro-
466 caudal axis of the NRe is innervated by catecholaminergic fibres, and that the LC provides a strong
467 catecholaminergic input to this nucleus. Interestingly the strongest innervation from LC appeared to
468 be to the rostral NRe. Next, optogenetic inactivation of LC→NRe significantly disrupted OiP retrieval,

but not encoding, while inactivation of the LC→HPC projection impaired encoding but not retrieval.

Finally, we found that retrieval was mediated by increased NA release in the NRe acting at α1-adrenoreceptors, while encoding required NA release in the HPC, specifically acting at β-adrenoceptors. Neither encoding nor retrieval appeared to depend on NA function in the mPFC. While NA release has been associated with attentional processing and arousal (Berridge, 2008; Berridge and Waterhouse, 2003; Sara, 2009; Schwarz and Luo, 2015), significantly none of the optogenetic or pharmacological experimental manipulations disrupted NOR or OL memory. That NOR was not affected is not that surprising given our previous work demonstrating that the HPC, NRe and mPFC are not involved in this form of recognition memory (Barker et al. 2011; 2018) although the lack of effect on OL does contrast with some earlier findings as will be discussed. Finally, overall object exploration during the sample or test phases was not affected by photostimulation or manipulation of noradrenergic receptor subtypes. Hence, we can exclude the possibility that the observed OiP deficits are due to nonspecific attentional or motivational deficits. Together these findings indicate the importance of NA neuromodulation in discrete brain regions for OiP memory encoding and retrieval.

The significant TH staining across the NRe observed, was found to be densest in rostral NRe. These results contrast with an earlier study which found that the catecholaminergic innervation of the midline nuclei, which includes NRe, is sparse (Lindvall et al., 1974). Such differences in findings are likely accounted for by different experimental protocols, as the earlier study used a glyoxylic acid fluorescence method, while here TH was used as the marker. TH is the rate-limiting step of catecholamine biosynthesis and therefore labels both dopaminergic and noradrenergic axons and while the present study did not distinguish neurochemical identity of these fibres, we revealed that the sole source of potential noradrenergic inputs to the NRe is the LC. However not all retrogradely labelled cells from the NRe to the LC were TH+ , thus the LC also likely sends non-catecholaminergic

494 inputs to the NRe which maybe either GABA-ergic or glutamatergic (Fung et al., 1994; Glennon et
495 al., 2019; Nakamura et al., 2000; Negishi et al., 2020; Yang et al., 2021; Ganley et al., 2021). Given
496 that our study was in no way meant to be a definitive anatomical investigation of inputs to NRe further
497 studies, including those investigating sources of dopaminergic inputs, are clearly needed. The finding
498 that the rostral NRe has the densest innervation of catecholaminergic fibres is potentially interesting
499 in the context of our previously described OiP memory network, as NRe→ HPC projections arise in
500 rostral NRe and projections to mPFC in caudal and lateral wings (Hoover and Vertes 2012; Varela et
501 al 2014). Clearly a next step would be to assess whether behaviour-specific patterns of neuronal
502 activity occur in HPC-projecting NRe cells modulated by NA.
503
504 We next focussed on the functional role of LC projections, and NA receptor subtypes in the HPC-
505 NRe-mPFC memory network. We consistently found that both disruption of NA signalling in the NRe
506 and disruption of LC input to NRe impaired associative recognition memory retrieval. Changes in
507 behavioural contingencies increase LC firing, thus signalling salience, novelty or
508 unexpected uncertainty (Vankov et al., 1995; Bouret and Sara, 2005; Yu and Dayan, 2005) as would
509 occur during the OiP test. While the effect of increased LC firing on NRe neurons has not been
510 investigated, in other thalamic nuclei such as the thalamic reticular nucleus, NA increases neuronal
511 excitability through α1-adrenoreceptor activation (McCormick and Prince, 1988; Lee and McCormick
512 1996). As associative memory retrieval requires activity in NRe→HPC pathway (Barker et al., 2021)
513 and the mPFC (Barker etal., 2007), it is tempting to speculate that recognition of a novel object-place
514 arrangement requires top down mPFC→ LC signalling of the object-place change (Schwarz et al.,
515 2015; Breton-Provencher and Sur, 2019) which results in increased LC firing, release of NA in NRe,
516 which acts via α1 adrenoreceptors located specifically on the NRe-hippocampal projection. Indeed,
517 it has been reported that some LC neurons project to a single brain area and thus have a selective

518 'modular' effect, (Kebschull et al., 2016) to optimise behavioural outcomes. NA release in the NRe
519 could thus act to promote on-going exploration of novelty (Beerling et al., 2011), as one would observe
520 in the OiP task, if retrieval was unaffected.

521

522 OiP encoding was disrupted by LC→HPC inhibition, agonism of α2-adrenergic receptors and
523 antagonism of β-adrenergic receptors. Previous research has shown that novelty, including that
524 during encoding of an object's new location, is associated with LC activation (Kempadoo et al., 2016;
525 Takeuchi et al., 2016; Gálvez-Márquez et al., 2022). Thus, it was surprising that LC→HPC inhibition
526 only impaired OiP and not OL memory which may reflect differences in task difficulty as the OL
527 requires a only single discrimination of the moved object. It has been shown that LC activation
528 releases NA in the HPC, leading to β-adrenergic dependent synaptic plasticity changes (Hansen and
529 Manahan-Vaughan, 2015; Hagena et al 2016; Babushkina and Manahan-Vaughan, 2022) specifically
530 long-term depression (Hagena and Manahan-Vaughan, 2025). Such plasticity could provide a
531 mechanism for the longer-term storage of object-place associative memories. However, some recent
532 studies have suggested that projections from the LC to the HPC release dopamine as well as NA and
533 it is the release of such dopamine, rather than NA which is critical for learning and memory (Kempadoo
534 et al., 2016; McNamara and Dupret, 2017; Takeuchi et al., 2016; Wagatsuma et al., 2017). However,
535 using the same protocols, we previously found that direct infusion of the D1/D5 antagonist SCH23390
536 into the HPC had no effect on OiP encoding (Savalli et al., 2015). Hence overall, our data indicating
537 that NA signalling in the HPC, via β-adrenergic receptors is required for OiP encoding may reflect the
538 involvement of a NA-mediated underlying long-term synaptic plasticity mechanism ensuring retention
539 of memory over a 3h delay.

540

Thus far encoding and retrieval have been discussed separately, although they are highly dynamic processes, and likely to be occurring, on most circumstances, at the same time, thus we used a modified version of OiP task involving two-test phases (Barker et al., 2021) and confirmed that LC→NRe inactivation impaired retrieval at Test 1, but did not impair encoding of the new information in Test 1, as Test 2 performance was intact. Conversely inactivation of the LC→HPC pathway impaired encoding but not retrieval. These results thus support our proposition that encoding and retrieval are mediated concurrently through separate but parallel LC-forebrain subnetworks, which may be key for the binding of recent and related information, whilst ensuring a separation of processing.

Surprisingly we found no effect of noradrenergic receptor manipulation in the mPFC although the mPFC is pivotal for associative recognition memory (Barker et al., 2007; Barker and Warburton, 2011; Benn et al., 2016), is strongly innervated by noradrenergic fibres, and has dense noradrenergic receptor expression (Santana et al., 2013; Rosin et al., 1996; Scheinin et al., 1994; Talley et al., 1996; Palacios and Kuhar, 1982; Paschalis et al., 2009). Interestingly those functional studies showing a critical role for NA in the mPFC have found effects on short-term working memory, attentional set shifting or in the extinction, but not acquisition of fear memory (see reviews in Berridge and Spencer, 2016; Mueller et al., 2008), thus, underlining the functional and regional specificity of LC and NA signalling in cognition. Indeed recent reports have argued that the LC is a heterogenous structure where separate populations of LC neurons send selective projections to provide this functional specificity (Borodovitsyna et al., 2020; Chandler et al., 2019; Chandler et al., 2014; Chandler and Waterhouse, 2012; Giustino et al., 2019; Hirschberg et al., 2017; Ranjbar-Slamloo and Fazlali, 2020; Totah et al., 2019; Uematsu et al., 2015; Uematsu et al., 2017).The present data clearly accord with this view i.e. that during associative recognition memory LC projections provide localised and hence modular neuromodulation in the NRe and HPC.

These findings demonstrate that memory encoding and retrieval are dependent both on activation of specific pathways and noradrenergic receptor subtypes within a hippocampal-thalamic memory circuit. Associative recognition memory deficits are associated with several neurodegenerative conditions and neuropsychiatric diseases such as schizophrenia (Crawford and Berry, 2024; Mäki-Marttunen et al., 2020). In ageing, LC cell number and NA concentration in the brain declines (Marien et al., 2004) and in both Parkinson's and Alzheimer's Disease LC degeneration occurs relatively early (Grudzien et al., 2007; Braak et al., 2004; Paredes-Rodriguez et al., 2020). Future work should consider a modular LC–NA system in the context of memory circuitry and prevention of memory decline.

578 **References**

579 Agster KL, Mejias-Aponte CA, Clark BD, Waterhouse BD (2013) Evidence for a regional specificity in
580 the density and distribution of noradrenergic varicosities in rat cortex. J Comp Neurol 521:2195–2207.

581

582 Atlas D, Steer ML, Levitzki A (1974) Stereospecific binding of propranolol and catecholamines to the
583 beta-adrenergic receptor. Proc Natl Acad Sci USA 71:4246–4248.

584

585 Babushkina N, Manahan‐Vaughan D (2022) Frequency–dependency of the involvement of
586 dopamine D1/D5 and beta‐adrenergic receptors in hippocampal LTD triggered by locus coeruleus
587 stimulation. Hippocampus 32: 449-465.

588

589 Banks PJ, Warburton EC, Bashir ZI (2021) Plasticity in prefrontal cortex induced by coordinated
590 synaptic transmission arising from reuniens/rhomboid nuclei and hippocampus. Cereb Cortex
591 Commun 2:1–16.

592

593 Barker GRI, Bird F, Alexander V, Warburton EC (2007) Recognition Memory for Objects, Place, and
594 Temporal Order: A disconnection analysis of the role of the medial prefrontal cortex and perirhinal
595 cortex. J Neurosci 27:2948–2957.

596

597 Barker GRI, Warburton EC (2011) When Is the Hippocampus Involved in Recognition Memory? J
598 Neurosci 31:10721–10731.

599

600 Barker GRI, Tran S, Gilroy K, Bashir ZI, Warburton EC (2021) Encoding and retrieval of associative
601 recognition memory engage different sub-networks within a hippocampal-thalamo-cortical memory
602 circuit. doi:10.21203/rs.3.rs-889826/v1

603

604 Barker GRI, Warburton EC (2011) When Is the Hippocampus Involved in Recognition Memory? J
605 Neurosci 31:10721–10731.

606

607 Barker GRI, Warburton EC (2018) A critical role for the nucleus reuniens in long-term, but not short-
608 term associative recognition memory formation J Neurosci 38:3208-3217

609

610 Beerling W, Koolhaas JM, Ahnaou A, Bouwknecht JA, de Boer SF, Meerlo P, Drinkenburg WH (2011)
611 Physiological and hormonal responses to novelty exposure in rats are mainly related to ongoing
612 behavioral activity. Physiol Behav 103:412-20.

613

614 Benn A, Barker GRI, Stuart SA, Roloff EL, Teschemacher AG, Warburton EC, Robinson ESJ (2016)
615 Optogenetic stimulation of prefrontal glutamatergic neurons enhances recognition memory. J
616 Neurosci 36:4930–4939.

617

618 Berridge CW (2008) Noradrenergic modulation of arousal. Brain Res Rev 58:1–17.

619

620 Berridge CW, Spencer RC (2016) Differential cognitive actions of norepinephrine a2 and a1 receptor
621 signaling in the prefrontal cortex. Brain Res 1641:189–196.

622

623 Berridge CW, Waterhouse BD (2003) The locus coeruleus-noradrenergic system: Modulation of
624 behavioral state and state-dependent cognitive processes. Brain Res Rev 42:33–84.

625

626 Borodovitsyna O, Duffy BC, Pickering AE, Chandler DJ (2020) Anatomically and functionally distinct
627 locus coeruleus efferents mediate opposing effects on anxiety-like behavior. Neurobiol Stress
628 13:100284.

629

630 Bouret S, Sara SJ (2005) Network reset: A simplified overarching theory of locus coeruleus
631 noradrenaline function. Trends Neurosci 28:574–582.

632

633 Boyajian CL, Loughlin SE, Leslie FM (1987) Anatomical evidence for alpha-2 adrenoceptor
634 heterogeneity : Differential autoradiographic distributions of [3H] rauwolscine and [3H] idazoxan in
635 rat brain. J Pharmacol Exp Ther 3:1079-1091.

636

637 Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K (2004) Stages in the development of
638 Parkinson's disease-related pathology. Cell Tissue Res, 318: 121-134.

639

640 Breton-Provencher V, Sur M (2019) Active control of arousal by a locus coeruleus GABAergic circuit.
641 Nat Neurosci 22:218-228

642

643 Bylund DB, Snyder SH (1976) Beta adrenergic receptor binding in membrane preparations from
644 mammalian brain. Mol Pharmacol 12:568–580.

645

646 Cerpa JC, Marchand AR, Coutureau E (2019) Distinct regional patterns in noradrenergic innervation
647 of the rat prefrontal cortex. J Chem Neuroanat 96:102–109.

648

649 Chandler DJ, Jensen P, McCall JG, Pickering AE, Schwarz LA, Totah NK (2019) Redefining
650 noradrenergic neuromodulation of behavior: Impacts of a modular locus coeruleus architecture. J
651 Neurosci 39:8239–8249.

652

653 Chandler DJ, Waterhouse BD (2012) Evidence for broad versus segregated projections from
654 cholinergic and noradrenergic nuclei to functionally and anatomically discrete subregions of prefrontal
655 cortex. Front Behav Neurosci 6:20.

656

657 Chandler DJ, Waterhouse BD, Gao WJ (2014) New perspectives on catecholaminergic regulation of
658 executive circuits: evidence for independent modulation of prefrontal functions by midbrain
659 dopaminergic and noradrenergic neurons. Front Neural Circuits 8: 53.

660

661 Crawford JL, Berry AS (2024) Examining resilience to Alzheimer's disease through the lens of
662 monoaminergic neuromodulator systems. Trends Neurosci 47:892-903

663

664 Dalley JW, Stanford SC (1995) Contrasting effects of the imidazol(in)e α2-adrenoceptor agonists,
665 medetomidine, clonidine and UK 14,304 on extra-neuronal levels of noradrenaline in the rat frontal -

cortex: evaluation using in vivo microdialysis and synaptosomal uptake studies. Br J Pharmacol 114:1717–1723.

Devoto, P., Flore, G., Saba, P., Fa, M. & Gessa, G. L.Co-release of noradrenaline and dopamine in thecerebral cortex elicited by single train and repeated train stimulation of the locus coeruleus. BMC Neurosci.6, 31 (2005).

Devoto P, Flore G, Pani L, Gessa GL (2001) Evidence for co-release of noradrenaline and dopamine fromnoradrenergic neurons in the cerebral cortex. Mol Psychiatry 6, 657–664

Dunsmoor JE, Murty VP, Clewett D, Phelps EA, Davachi L (2022) Tag and capture: how salient experiences target and rescue nearby events in memory. Trends Cog Sci 26: 782-795.

Fan X, Song J, Ma C, Lv Y, Wang F, Ma L, Liu X (2022) Noradrenergic signaling mediates cortical early tagging and storage of remote memory. Nature Comm 13: 7623.

Fernández-Pastor B, Meana JJ (2002) In vivo tonic modulation of the noradrenaline release in the rat cortex by locus coeruleus somatodendritic α2-adrenoceptors. Eur J Pharmacol 442:225–229.

Ferry B, Parrot S, Marien M, Lazarus C, Cassel JC, McGaugh JL (2015) Noradrenergic influences in the basolateral amygdala on inhibitory avoidance memory are mediated by an action on α2-adrenoceptors. Psychoneuroendocrinology 51:68–79.

Fukabori R, Iguchi Y, Kato S, Takahashi K, Eifuku S, Tsuji S, Hazama A, Uchigashima M, Watanabe M, Mizuma H, Cui Y (2020) Enhanced retrieval of taste associative memory by chemogenetic activation of locus coeruleus norepinephrine neurons. J Neurosci, 40:8367-8385.

Fung SJ, Reddy VK, Liu RH, Wang Z, Barnes CD (1994) Existence of glutamate in noradrenergic locus coeruleus neurons of rodents. Brain Res Bull 35:505–512.

Fuxe, D. (1964). Evidence for existence of monoamine-containing neurons in central nervous system. i. demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand., 62, 1–55.

Gálvez-Márquez DK, Salgado-Ménez M, Moreno-Castilla P, Rodríguez-Durán L, Escobar ML, Tecuapetla F, Bermudez-Rattoni F (2022) Spatial contextual recognition memory updating is modulated by dopamine release in the dorsal hippocampus from the locus coeruleus. Proc Nat Acad Sci USA 119: e2208254119.

Ganley RP, Werder K, Wildner H, Zeilhofer HU (2021) Spinally projecting noradrenergic neurons of the locus coeruleus display resistance to AAV2retro-mediated transduction. Mol Pain 17:17448069211037887.

Giustino TF, Fitzgerald PJ, Ressler RL, Maren S (2019) Locus coeruleus toggles reciprocal prefrontal firing to reinstate fear. Proc Natl Acad Sci U S A 116:8570–8575.

711

712 Glennon E, Carcea I, Martins ARO, Multani J, Shehu I, Svirsky MA, Froemke RC (2019) Locus
713 coeruleus activation accelerates perceptual learning. Brain Res 1709:39–49.

714

715 Greengrass P, Bremmer R (1979) Binding characteristics of 3H-prazosin to rat brain α-adrenergic
716 receptors. Eur J Pharmacol 55:323–326.

717

718 Grudzien A, Shaw P, Weintraub S, Bigio E, Mash DC, Mesulam MM (2007) Locus coeruleus
719 neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease.
720 Neurobiol Aging 28: 327-335.

721

722 Hagena H, Hansen N, Manahan-Vaughan D (2016) β-adrenergic control of hippocampal function:
723 subserving the choreography of synaptic information storage and memory. Cereb Cortex 26:1349–
724 1364.

725

726 Hagena H, Manahan-Vaughan D. (2025) Oppositional and competitive instigation of hippocampal
727 synaptic plasticity by the VTA and locus coeruleus. Proc Natl Acad Sci U S A. 2025
728 122(1):e2402356122.

729

730 Hansen N, Manahan-Vaughan D (2015) Locus coeruleus stimulation facilitates long-term depression
731 in the dentate gyrus that requires activation of β-adrenergic receptors. Cereb Cortex 25:1889–1896.

732

733 Hirschberg S, Li Y, Randall A, Kremer EJ, Pickering AE (2017) Functional dichotomy in spinal-vs
734 prefrontal-projecting locus coeruleus modules splits descending noradrenergic analgesia from
735 ascending aversion and anxiety in rats. Elife 6:1–26.

736

737 Hokfelt, T. (1984). Distribution maps of tyrosine hydroxylase-immunoreactive neurons in the rat
738 brain. Handbook of Chemical Neuroanatomy, Classical Transmitters in the CNS, 2, 277–379.

739

740 Hoover WB, Vertes RP (2012) Collateral projections from nucleus reuniens of thalamus to
741 hippocampus and medial prefrontal cortex in the rat: a single and double retrograde. Brain Struct
742 Funct. 217:191-209

743

744 Horrillo I, Ortega JE, Diez-Alarcia R, Urigüen L, Meana JJ (2019) Chronic fluoxetine reverses the
745 effects of chronic corticosterone treatment on α2-adrenoceptors in the rat frontal cortex but not locus
746 coeruleus. Neuropharmacology 158:107731.

747

748 Kebschull JM, Garcia da Silva P, Reid AP, Peikon ID, Albeanu DF, Zador AM (2016) High-
749 throughput mapping of single-neuron projections by sequencing of barcoded RNA. Neuron 91:975–
750 987.

751

752 Kempadoo KA, Mosharov EV, Choi SJ, Sulzer D, Kandel ER (2016) Dopamine release from the
753 locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. Proc Natl Acad
754 Sci U S A 113:14835–14840.

755

Langer SZ (1974) Presynaptic regulation of catecholamine release. Biochem Pharmacol 23:1793–1800.

Lee KH, McCormick DA (1996) Abolition of spindle oscillations by serotonin and norepinephrine in the ferret lateral geniculate and perigeniculate nuclei in vitro. Neuron, 17: 309-321.

Lefkowitz RJ, Limbird LE, Mukherjee C, Caron MG (1976) The ß-adrenergic receptor and adenylate cyclase. BBA - Rev Biomembr 457:1–39.

Lemon N, Aydin-Abidin S, Funke K, Manahan-Vaughan D (2009) Locus coeruleus activation facilitates memory encoding and induces hippocampal LTD that depends on β-Adrenergic receptor activation. Cereb Cortex 19:2827–2837.

Lindvall O, Björklund A, Nobin A, Stenevi U (1974) The adrenergic innervation of the rat thalamus as revealed by the glyoxylic acid fluorescence method. J Comp Neurol 154:317–347.

Loughlin SE, Foote SL, Bloom FE (1986) Efferent projections of nucleus locus coeruleus: Topographic organization of cells of origin demonstrated by three-dimensional reconstruction. Neuroscience 18:291–306.

Mäki-Marttunen V, Andreassen OA, Espeseth T (2020) The role of norepinephrine in the pathophysiology of schizophrenia. Neurosci Biobehav Rev 118:298-314.

Marien MR, Colpaert FC, Rosenquist AC (2004) Noradrenergic mechanisms in neurodegenerative diseases: a theory. Brain Res Rev, 45: 38-78.

McCormick DA, Prince DA (1988) Noradrenergic modulation of firing pattern in guinea pig and cat thalamic neurons, in vitro. J Neurophysiol 59:978–996.

McKenna JT, Vertes RP (2004) Afferent projections to nucleus reuniens of the thalamus. J Comp Neurol 480:115–142.

McNamara CG, Dupret D (2017) Two sources of dopamine for the hippocampus. Trends Neurosci 40:383–384.

Mello-Carpes PB, da Silva de Vargas L, Gayer MC, Roehrs R, Izquierdo I (2016) Hippocampal noradrenergic activation is necessary for object recognition memory consolidation and can promote BDNF increase and memory persistence. Neurobiol Learn Mem 127:84–92.

Milner TA, Lee A, Aicher SA, Rosin DL (1998) Hippocampal α(2A)-adrenergic receptors are located predominantly pre-synaptically but are also found post-synaptically and in selective astrocytes. J Comp Neurol 395:310–327.

799 Mueller D, Porter JT, Quirk GJ (2008) Noradrenergic signaling in infralimbic cortex increases cell
800 excitability and strengthens memory for fear extinction. J Neurosci, 28: 369-375.

801

802 Nakamura Y, Kitani T, Okuno S, Otake K, Sato F, Fujisawa H (2000) Immunohistochemical study of
803 the distribution of Ca2+/calmodulin- dependent protein kinase phosphatase in the rat central
804 nervous system. Mol Brain Res 77:76–94.

805

806 Negishi K, Payant MA, Schumacker KS, Wittmann G, Butler RM, Lechan RM, Steinbusch HW, Khan
807 AM, Chee MJ (2020) Distributions of hypothalamic neuron populations co-expressing tyrosine
808 hydroxylase and the vesicular GABA transporter in the mouse. J Comp Neurol, 528: 1833-1855.

809

810 Palacios JM, Kuhar MJ (1982) Beta adrenergic receptor localization in rat brain by light microscopic
811 autoradiography. Neurochem Int 4:473–490.

812

813 Paredes-Rodriguez E, Vegas-Suarez S, Morera-Herreras T, De Deurwaerdere P, Miguelez C
814 (2020) The noradrenergic system in Parkinson's disease. Front Pharmacol, 11, 435.

815

816 Paschalis A, Churchill L, Marina N, Kasymov V, Gourine A, Ackland G (2009) β1-Adrenoceptor
817 distribution in the rat brain: An immunohistochemical study. Neurosci Lett 458:84–88.

818

819 Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. Elsevier.

820

821 Ranjbar-Slamloo Y, Fazlali Z (2020) Dopamine and noradrenaline in the brain; overlapping or
822 dissociate functions? Front Mol Neurosci 12:1–8.

823

824 Rosin DL, Talley EM, Lee AMY, Stornetta RL, Gaylinn BD, Guyenet PG, Lynch KR (1996)
825 Distribution of α2C - adrenergic receptor-like immunoreactivity in the rat central nervous system.
826 165:135–165.

827

828 Santana N, Mengod G, Artigas F (2013) Expression of α1-adrenergic receptors in rat prefrontal
829 cortex: Cellular co-localization with 5-HT2A receptors. Int J Neuropsychopharmacol 16:1139–1151.

830

831 Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci
832 10:211–223.

833

834 Sara SJ, Vankov A, Hervé A (1994) Locus coeruleus-evoked responses in behaving rats: A clue to
835 the role of noradrenaline in memory. Brain Res Bull 35:457–465.

836

837 Sargent-Jones L, Gauger LL, Davis JN (1985) Anatomy of brain alpha-1 adrenergic receptors: in
838 vitro autoradiography with $^{[125]}$HEAT. J Comp Neurol 231:190–208.

839

840 Savalli G, Bashir ZI, Warburton EC (2015) Regionally selective requirement for D 1 /D 5
841 dopaminergic neurotransmission in the medial prefrontal cortex in object-in-place associative
842 recognition memory. Learn Mem 22:69–73.

Scheinin M, Lomasney JW, Hayden-Hixson DM, Schambra UB, Caron MG, Lefkowitz RJ, Fremeau RT (1994) Distribution of α2-adrenergic receptor subtype gene expression in rat brain. Mol Brain Res 21:133–149.

Schwarz LA, Luo L (2015) Organization of the locus coeruleus-norepinephrine system. Curr Biol 25:R1051-R1056.

Schwarz LA, Miyamichi K, Gao XJ, Beier KT, Weissbourd B, Deloach KE, Ren J, Ibanes S, Malenka RC, Kremer EJ, Luo L (2015) Viral-genetic tracing of the input-output organisation of a central noradrenaline circuit. Nature 524:88-92

Smith CC, Greene RW (2012) CNS dopamine transmission mediated by noradrenergic innervation. J Neurosci 32:6072–6080.

Starke K (2001) Presynaptic autoreceptors in the third decade: Focus on α2-adrenoceptors. J Neurochem 78:685–693.

Swanson LW, Hartman BK (1975) The central adrenergic system. An immunohistofluorescence study of the location of cell bodies and their efferent connections in the rat using dopamine-b-hydroxylase as a marker. J Comp Neurol 163:467–506.

865

866 Takeuchi T, Duszkiewicz AJ, Sonneborn A, Spooner PA, Yamasaki M, Watanabe M, Smith CC,
867 Fernández G, Deisseroth K, Greene RW, Morris RGM (2016) Locus coeruleus and dopaminergic
868 consolidation of everyday memory. Nature 537:357–362.

869

870 Talley EM, Rosin DL, Lee AMY, Guyenet PG, Lynch KR (1996) Distribution of -adrenergic receptor-
871 like immunoreactivity in the rat central nervous system. J Comp Neurol 134:135–165.

872

873 Torkaman-Boutorabi A, Danyali F, Oryan S, Ebrahimi-Ghiri M, Zarrindast MR (2014) Hippocampal
874 α-adrenoceptors involve in the effect of histamine on spatial learning. Physiol Behav 129:17–24.

875

876 Totah NKB, Logothetis NK, Eschenko O (2019) Noradrenergic ensemble-based modulation of
877 cognition over multiple timescales. Brain Res 1709:50–66.

878

879 Tsetsenis T, Badyna JK, Li R, Dani JA (2022) Activation of a locus coeruleus to dorsal hippocampus
880 noradrenergic circuit facilitates associative learning. Front Cell Neurosci, 16, 887679.

881

882 U'Prichard DC, Charness ME, Robertson D, Snyder SH (1978) Prazosin: differential affinities for two
883 populations of α-noradrenergic receptor binding sites. Eur J Pharmacol 50:87–89.

884

885 Uematsu A, Tan BZ, Johansen JP (2015) Projection specificity in heterogeneous locus coeruleus cell
886 populations: Implications for learning and memory. Learn Mem 22:444–451.

887

888 Uematsu A, Tan BZ, Ycu EA, Cuevas JS, Koivumaa J, Junyent F, Kremer EJ, Witten IB, Deisseroth
889 K, Johansen JP (2017) Modular organization of the brainstem noradrenaline system coordinates
890 opposing learning states. Nat Neurosci 20:1602–1611.

891

892 van Meel JC, de Jonge A, Timmermans PB, van Zwieten PA (1981) Selectivity of some alpha
893 adrenoceptor agonists for peripheral alpha-1 and alpha-2 adrenoceptors in the normotensive rat. J
894 Pharmacol Exp Ther 219:760–767.

895

896 van Veldhuizen MJ, Feenstra MG, Heinsbroek RP, Boer GJ (1993) In vivo microdialysis of
897 noradrenaline overflow: effects of α-adrenoceptor agonists and antagonists measured by cumulative
898 concentration-response curves. Br J Pharmacol 109:655–660.

899

900 Vankov A, Hervé-Minvielle A, Sara SJ (1995) Response to novelty and its rapid habituation in locus
901 coeruleus neurons of the freely exploring rat. Eur J Neurosci 7:1180–1187.

902

903 Varela C, Kumar S, Yang JY, Wilson MA (2014) Anatomical substrates for direct interactions between
904 hippocampus, medial prefrontal cortex, and the thalamic nucleus reuniens. Brain Struct Funct 219:
905 911–929.

906

907 Wagatsuma A, Okuyama T, Sun C, Smith LM, Abe K, Tonegawa S (2017) Locus coeruleus input to
908 hippocampal CA3 drives single-trial learning of a novel context. Proc Natl Acad Sci U S A 115: E310–
909 E316.

910

911 Waterhouse BD, Chandler D (2012) Evidence for broad versus segregated projections from
912 cholinergic and noradrenergic nuclei to functionally and anatomically discrete subregions of prefrontal
913 cortex. Front Behav Neurosci 6:1–9.

914

915 Wilson LR., Plummer NW, Evsyukova IY, Patino D, Stewart CL, Smith KG, Konrad KS, Fry SA, Deal
916 AL, Kilonzo VW Panda S (2024) Partial or complete loss of norepinephrine differentially alters
917 contextual fear and catecholamine release dynamics in hippocampal CA1. Biol Psychiatry Glob Open
918 Sci 4: 51-60.

919

920 Yang B, Sanches-Padilla J, Kondapalli J, Morison SL, Delpire E, Awatramani R, Surmeier DJ (2021)
921 Locus coeruleus anchors a trisynaptic circuit controlling fear-induced suppression of feeding. Neuron
922 109:823-838.e6.

923

924 Yu AJ, Dayan P (2005) Uncertainty, neuromodulation, and attention. Neuron 46:681-92

925

926

927

928

929

930 **Figure Legends**

931

932 **Figure 1 Origin of catecholaminergic input to the NRe.**

933 **A-C** Distribution of TH-positive fibres in the NRe. (Left panel) Schematic of the Paxinos and Watson
934 (2007) brain atlas at three anterior-posterior levels with highlighted area (dashed black box) indicating
935 region in which photomicrographs were taken and distance in mm from bregma. (Middle panel)
936 Representative photomicrographs of TH immunoreactive fibres in the thalamus. (Right panel) High-
937 magnification photomicrographs of region indicated by boxes in middle panel.

938 **D** Schematic drawings of retrograde tracer injection spread in each case. Each individual case is
939 colour coded, and the numbers #1-#7 correspond to rostral-caudal injection sites (see Table 1).

940 **E** Representative case #3 showing spread of CTB tracer in the NRe.

941 **F** Fluorescent photomicrographs of case #1 showing retrogradely transported FB neurons (blue), TH-
942 positive neurons (green) and an overlay of the two images in the LC. Double labelled neurons
943 highlighted by the white arrowheads.

944 **G** Proportion of double-labelled neurons (grey) relative to the number of retrogradely transported cells
945 (green) in A6 for each case. Raw numbers are in brackets and percentages show the proportion of
946 double-labelled neurons following the injections at different levels (Table 1).Scale bars: 200µm.

947 Abbreviations: A11, A11 dopamine cells; A13, A13 dopamine cells; AHP, anterior

948 hypothalamic area, posterior part; AM, anteromedial thalamic nucleus; AMV, anteromedial

949 thalamic nucleus, ventral part; ANS, accessory neurosecretory nuclei; CM, central medial

950 thalamic nucleus; DA, dorsal hypothalamic area; DMD, dorsomedial hypothalamic nucleus,

951 dorsal part; IAD, interanterodorsal thalamic nucleus; IAM, interanteromedial thalamic

952 nucleus; JLPH, juxtaparaventricular part of lateral hypothalamus; MT, medial terminal

nucleus of the accessory optic tract; PaDC, paraventricular hypothalamic nucleus, dorsal cap; PaLM, paraventricular hypothalamic nucleus, lateral magnocellular part; PaMP, paraventricular hypothalamic nucleus, medial parvicellular part; PaXi, paraxiphoid nucleus of thalamus; Pe, periventricular hypothalamic nucleus; PH, posterior hypothalamic nucleus; PHD, posterior hypothalamic area, dorsal part; PT, paratenial thalamic nucleus; PVA, paraventricular thalamic nucleus, posterior part; Re, reuniens thalamic nucleus; Rh, rhomboid thalamic nucleus; Stg, stigmoid hypothalamic nucleus; Sub, submedius thalamic nucleus; SubD, submedius thalamic nucleus, dorsal part; SubV, submedius thalamic nucleus, ventral part; VM, ventromedial thalamic nucleus; VRe, ventral reuniens thalamic nucleus; Xi, xiphoid thalamic nucleus. Figures adapted from Paxinos and Watson (2007).

Figure 2 Differential effects of inhibition of LC→NRe and LC→HPC projections on encoding and retrieval of object-in-place, novel object recognition and object location memory

A Schematic of experimental approach for in vivo optogenetic inhibition.

B Representative image of viral expression in the LC.

C Representative image in the NRe showing optrode tracts (left) and Arch3.0-EYFP expression (right).

D Representative image in the HPC showing optrode tracts (left) and Arch3.0-EYFP expression (right).

E OiP performance following light delivery into the NRe and HPC, in the Arch (n=12) and YFP (n=12) animals during the sample phase compared to a no-light 'Off' condition.

F OiP performance following light delivery into the NRe and HPC, in the Arch (n=12) and YFP (n=12) animals during the test phase compared to a no-light 'Off' condition.

G OiP performance in the Arch (n=12) and YFP (n=12) animals, in test phase 1 and test phase 2 with light delivery into the NRe and HPC during test phase 1.

H NOR performance following light delivery into the NRe and HPC, in the Arch (n=12) and YFP (n=12) animals during the sample phase compared to a no-light condition (all F<1 n.s.).

I NOR performance following light delivery into the NRe and HPC, in the Arch (n=12) and YFP (n=12) animals during the test phase compared to a no-light condition (all F<1 n.s.).

J OL performance following light delivery into the NRe and HPC, in the Arch (n=12) and YFP (n=12) animals during the sample phase compared to a no-light condition (all F<1 n.s.).

J Object location performance following light delivery into the NRe and HPC, in the Arch (n=12) and YFP (n=12) animals during the test phase compared to a no-light condition (all F<1 n.s.).

All data represented as means ± SEM and circles represent individual animals. * p<0.05; ** p<0.01 difference between groups. Scale bars: 200µm.

Figure 3 The differential role of adrenergic receptors on OiP encoding and retrieval

A and B Schematic of experimental approach for intracerebral administration of specific adrenergic receptor agonists/antagonists.

C Schematic representation of the OiP task.

D The effects of intra-NRe administration of prazosin or propranolol prior to the sample phase or test phase. (n = 10).

E The effects of intra-HPC or intra-mPFC infusion of prazosin prior to the sample or test phase, (n = 12).

F The effect of administration of propranolol into the HPC, or mPFC before the sample or test phase (n = 11).

G The effect of administration of UK 14,304 into the NRe before the sample or test phase (n = 10).

H The effect of administration of UK 14,304 into the HPC or PFC either before the sample or test phase.

I The effect of administration of RS79948 in the NRe before the sample or test.

J The effect of RS 79948 into the HPC or mPFC before the sample or test

Data represented as means ± SEM and circles represent individual animals, * $p < .05$, ** $p < .01$, *** $p<0.001$

Table 1. Overview of individual cases with details of retrograde tracers used and method of injection

Case	Tracer	Co-ordinates	Method of injection	Main site of tracer deposit
#1	FB	AP: -1.9, ML: sinus, DV: -7.4	Pressure	Rostral NRe
#2	CTB	AP: -1.9, ML: sinus, DV: -7.5	Pressure	Rostral NRe
#3	CTB	AP: -1.9, ML: sinus, DV: -7.5	Pressure	Rostral NRe
#4	CTB	AP: -1.9, ML: sinus, DV: -6.8 (dura)	Iontophoretic	Rostral NRe
#5	CTB	AP: -2.4, ML: sinus, DV: -6.9 (dura)	Iontophoretic	Intermediate to caudal NRe
#6	FG	AP: -2.6, ML: sinus, DV: -6.8 (dura)	Iontophoretic	Intermediate to caudal NRe
#7	CTB	AP: -2.6, ML: sinus, DV: -6.7 (dura)	Iontophoretic	Intermediate to caudal NRe

Table 2. Mean exploration times ± SEM in the sample and test phases of animals involved in optogenetic experiments

Figure and task	Stimulation timing	Virus	Stimulation condition	Exploration in sample phase (s)	Statistical analysis of sample phase	Exploration in test phase 1 (s)	Statistical analysis of test phase 1	Exploration in test phase 2 (s)	Statistical analysis of test phase 2
Figure 2E and 2F Object-in-place	Encoding	YFP	Off	57.9 ± 4.18	Stimulation condition x virus (F(2,44) = 2.70, p = .079) Main effect of stimulation condition (F(2,44) = 1.87, p = .166) Main effect of virus (F(1,22) = .831, p = .372)	32.0 ± 4.10	Stimulation condition x virus (F(1.54,33.9) = .572, p = .526) Main effect of stimulation condition (F(1.54,33.9) = 1.69, p = .204) Main effect of virus (F(1,22) = .124, p = .728)		
			NRe	49.7 ± 4.76		28.2 ± 3.16			
			HPC	41.9 ± 2.52		28.7 ± 3.03			
		Arch	Off	45.5 ± 3.90		31.1 ± 3.29			
			NRe	47.5 ± 3.52		29.9 ± 1.98			
			HPC	47.0 ± 4.29		24.6 ± 3.01			
	Retrieval	YFP	Off	71.5 ± 5.54	Stimulation condition x virus (F(2,44) = .687,p = .508) Main effect of stimulation condition (F(2,44) = .146, p = .865) Main effect of virus (F(1,22) = .907, p = .351)	31.8 ± 4.17	Stimulation condition x virus (F(2,44) = 1.11, p = .338) Main effect of stimulation condition (F(2,44) = .438, p = .648) Main effect of virus (F(1,22) = .004, p = .950)		
			NRe	68.0 ± 5.34		33.9 ± 4.28			
			HPC	66.3 ± 3.55		28.8 ± 3.28			
		Arch	Off	62.3 ± 4.15		32.6 ± 2.77			
			NRe	65.1 ± 4.39		29.7 ± 3.22			
			HPC	64.1 ± 3.00		31.4 ± 3.48			
Figure 2G Object-in-place (two test phases)		YFP	Off	71.0 ± 4.36	Stimulation condition x virus (F(2,44) = .006, p = .994) Main effect of stimulation condition (F(2,44) = .273, p = .762) Main effect of virus (F(1,22) = .166, p = .688)	48.4± 4.40	Stimulation condition x virus (F(2,44) = .184, p = .833) Main effect of stimulation condition (F(2,44) = .014, p = .986) Main effect of virus (F(1,22) = 3.66, p = .069)	34.9 ± 2.22	Stimulation condition x virus (F(2,44) = .663, p = .521) Main effect of stimulation condition (F(2,44) = .006, p = .994) Main effect of virus (F(1,22) = .232, p = .635)
			NRe	72.5 ± 5.37		47.8 ± 4.49		38.6 ± 3.41	
			HPC	69.8 ± 6.41		45.6 ± 3.54		37.4 ± 2.71	
		Arch	Off	68.2 ± 5.71		55.3 ± 5.49		37.6 ± 3.83	
			NRe	70.6 ± 3.42		54.5 ± 5.35		34.3 ± 1.92	
			HPC	67.6 ± 4.74		56.8 ± 4.33		35.9 ± 3.20	
Figure 2H and 2I Object recognition	Encoding	YFP	Off	62.7 ± 3.62	Stimulation condition x virus (F(2,42) = .041, p = .959) Main effect of stimulation condition (F(2,42) = 1.84, p = .171) Main effect of virus (F(1,21) = .005, p = .947)	36.9 ± 2.81	Stimulation condition x virus (F(2,42) = .671, p = .517) Main effect of stimulation condition (F(2,42) = 1.77, p = .183) Main effect of virus (F(1,21) = 1.05, p = .318)		
			NRe	64.8 ± 2.59		41.8 ± 2.90			
			HPC	59.4 ± 3.13		36.0 ± 2.75			
		Arch	Off	61.3 ± 5.26		36.3 ± 3.08			
			NRe	63.2 ± 3.05		41.8 ± 5.23			
			HPC	59.1 ± 4.52		43.1 ± 2.19			
	Retrieval	YFP	Off	67.2 ± 4.99	Stimulation condition x virus (F(2,44) = .919, p = .407) Main effect of stimulation condition (F(2,44) = 2.95, p = .063) Main effect of virus (F(1,22) = .033, p = .858)	39.6 ± 3.11	Stimulation condition x virus interaction (F(2,44) = .035, p = .966) Main effect of stimulation condition (F(2,44) = .623, p = .541) Main effect of virus (F(1,22) = .869, p = .361)		
			NRe	79.5 ± 3.95		35.0 ± 4.33			
			HPC	72.0 ± 4.82		36.9 ± 4.04			
		Arch	Off	69.5 ± 5.31		43.0 ± 3.33			
			NRe	74.8 ± 4.03		39.5 ± 4.71			
			HPC	77.0 ± 4.08		39.4 ± 4.61			
Figure 2J and 2K Object location	Encoding	YFP	Off	39.8 ± 3.74	Stimulation condition x virus (F(2,44) = .828, p = .444) Main effect of stimulation condition (F(2,44) = .066, p = .936) Main effect of virus (F(1,22) = .006, p = .937)	27.3 ± 3.05	Stimulation condition x virus interaction (F(2,44) = .047, p = .954) Main effect of stimulation condition (F(2,44) = .098, p = .907) Main effect of virus(F(1,22) = 4.08, p = .056)		
			NRe	36.8 ± 4.13		26.5 ± 2.51			
			HPC	39.0 ± 4.07		25.7 ± 2.72			
		Arch	Off	37.2 ± 1.81		31.6 ± 2.76			
			NRe	40.1 ± 3.06		32.1 ± 2.64			
			HPC	39.3 ± 3.19		31.3 ± 2.03			
	Retrieval	YFP	Off	39.8 ± 4.46	Stimulation condition x virus (F(2,44) = .268, p = .766) Main effect of stimulation condition (F(2,44) = .662, p = .521) Main effect of virus (F(1,22) = .011, p = .919)	28.4 ± 2.93	Stimulation condition x virus (F(2,44) = .753, p = .872) Main effect of stimulation condition (F(2,44) = .027, p = .974) Main effect of virus (F(1,22) = .083, p = .775)		
			NRe	41.2 ± 4.17		28.2 ± 3.85			
			HPC	38.9 ± 2.71		30.3 ± 2.40			
		Arch	Off	38.2 ± 2.83		28.2 ± 2.36			
			NRe	42.3 ± 3.19		29.3 ± 2.74			
			HPC	40.8 ± 3.64		25.6 ± 2.69			

Table 3. Analysis of performance against chance of animals involved in optogenetic experiment

Figure and task	Stimulation timing	Virus	Stimulation condition	Statistical analysis comparing performance against chance in test phase 1	Statistical analysis comparing performance against chance in test phase 2
Figure 2E and 2F Object-in-place	Encoding	YFP	Off	t(11) = 7.28, p < .001	
			NRe	t(11) = 4.17, p = .002	
			HPC	t(11) = 3.95, p = .002	
		Arch	Off	t(11) = 3.44, p = .006	
			NRe	t(11) = 5.63, p < .001	
			HPC	t(11) = -.425, p = .679	
	Retrieval	YFP	Off	t(11) = 5.30, p < .001	
			NRe	t(11) = 7.12, p < .001	
			HPC	t(11) = 5.35, p < .001	
		Arch	Off	t(11) = 4.66, p = .001	
			NRe	t(11) = .704, p = .496	
			HPC	t(11) = 3.66, p = .004	
Figure 2G Object-in-place (two test phases)		YFP	Off	t(11) = 5.52, p < .001	t(11) = 3.59, p = .004
			NRe	t(11) = 4.17, p = .001	t(11) = 3.63, p = .004
			HPC	t(11) = 3.95, p = .001	t(11) = 4.22, p = .001
		Arch	Off	t(11) = 6.54, p < .001	t(11) = 4.35, p = .001
			NRe	t(11) = -.853, p = .412	t(11) = 4.20, p = .001
			HPC	t(11) = 7.97, p < .001	t(11) = -2.49, p = .808
Figure 2H and 2I Object recognition	Encoding	YFP	Off	t(11) = 4.59, p = .001	
			NRe	t(11) = 4.18, p = .002	
			HPC	t(11) = 4.40, p = .001	
		Arch	Off	t(11) = 3.72, p = .003	
			NRe	t(11) = 5.27, p < .001	
			HPC	t(10) = 3.31, p = .008	
	Retrieval	YFP	Off	t(11) = 4.22, p = .001	
			NRe	t(11) = 4.04, p = .002	
			HPC	t(11) = 4.21, p = .001	
		Arch	Off	t(11) = 3.35, p = .007	
			NRe	t(11) = 4.62, p = .001	
			HPC	t(11) = 4.11, p = .002	
Figure 2J and 2K Object location	Encoding	YFP	Off	t(11) = 2.93, p = .014	
			NRe	t(11) = 3.44, p = .006	
			HPC	t(11) = 3.67, p = .004	
		Arch	Off	t(11) = 4.08, p = .002	
			NRe	t(11) = 6.10, p < .001	
			HPC	t(11) = 2.97, p = .013	
	Retrieval	YFP	Off	t(11) = 6.23, p < .001	
			NRe	t(11) = 4.60, p = .001	
			HPC	t(11) = 4.11, p = .002	
		Arch	Off	t(11) = 3.75, p = .003	
			NRe	t(11) = 4.19, p = .002	
			HPC	t(11) = 3.35, p = .006	

Table 4. Mean exploration times ± SEM in the sample and test phases of NRe-infused animals

Figure	Infusion timing	Condition	Exploration in sample phase (s)	Statistical analysis of sample phase	Exploration in test phase (s)	Statistical analysis of test phase
Figure 3D Prazosin and propranolol	Pre-sample	Vehicle	74.2 ± 10.7	Drug x infusion timing (F(2,36) = .085, p = .918) Main effect of drug (F(2,36)= 1.24, p = .301) *Main effect of infusion timing (F(1,18) = 6.00,p = .025)	47.5 ± 5.29	Drug x infusion timing (F(2,36) = .268, p = .766) Main effect of drug (F(2,36)= .295, p = .747) Main effect of infusion timing (F(1,18)= .267, p = .612)
		Prazosin	83.2 ± 6.40		47.5 ± 7.90	
		Propranolol	84.7 ± 6.30		49.5 ± 6.48	
	Pre-test	Vehicle	89.7 ± 4.37		41.7 ± 2.54	
		Prazosin	92.9 ± 7.74		48.2 ± 6.12	
		Propranolol	99.6 ± 3.46		45.4 ± 3.91	
Figure 3G UK 14,304	Pre-sample	Vehicle	76.9 ± 6.05	Drug x infusion timing (F(1,18) = .516, p = .482) Main effect of drug (F(1,18)= .062, p = .806) Main effect of infusion timing (F(1,18) =4.02, p = .060)	38.2 ± 3.23	Drug x infusion timing (F(1,18) = .026, p = .874) Main effect of drug (F(1,18) = .203, p = .658) *Main effect of infusion timing (F(1,18) = 4.75, p = .043)
		UK 14,304	79.3 ± 7.37		37.1 ± 3.29	
	Pre-test	Vehicle	93.8 ± 4.68		51.6 ± 5.71	
		UK 14,304	88.8 ± 5.05		46.4 ± 6.42	
Figure 3I RS79488	Pre-sample	Vehicle	85.5 ± 5.19	Drug x infusion timing (F(1,16) = .293, p = .596) Main effect of drug (F(1,16)= 1.89, p = .188) *Main effect of infusion timing (F(1,16) = 10.3, p = .006)	50.5 ± 4.79	Drug x infusion timing (F(1,16) = .521, p = .481) Main effect of drug (F(1,16)= .001, p = .982) Main effect of infusion timing (F(1,16) = 3.33, p = .087)
		RS79488	76.5 ± 10.4		48.3 ± 7.33	
	Pre-test	Vehicle	59.2 ± 4.44		44.6 ± 2.77	
		RS79488	54.7 ± 4.57		42.0 ± 3.27	

Table 5. Analysis of performance against chance of NRe-infused animals

Figure	Infusion timing	Condition	Statistical analysis of sample phase
Figure 3D Prazosin and propranolol	Pre-sample	Vehicle	t(9) = -4.10, p = .003
		Prazosin	t(9) = 4.48, p = .001
		Propranolol	t(9) = 3.17, p = .011
	Pre-test	Vehicle	t(9) = 3.10, p = .013
		Prazosin	t(9) = -1.70, p = .123
		Propranolol	t(9) = 3.66, p = .005
Figure 3G UK 14,304	Pre-sample	Vehicle	t(9) = 5.05, p = .001
		UK 14,304	t(9) = 3.64, p = .005
	Pre-test	Vehicle	t(9) = 4.32, p = .002
		UK 14,304	t(9) = .092, p = .928
Figure 3I RS79488	Pre-sample	Vehicle	t(8) = 4.17, p = .003
		RS79488	t(8) = 5.25, p = .001
	Pre-test	Vehicle	t(8) = 3.47, p = .008
		RS79488	t(8) = 4.44, p = .002

Table 6. Mean exploration times ± SEM in the sample and test phases of HPC or mPFC-infused animals

Figure and drug	Infusion timing	Drug condition	Exploration in sample phase (s)	Statistical analysis of sample phase	Exploration in test phase (s)	Statistical analysis of test phase
Figure 3E Prazosin	Pre-sample	Vehicle	87.0 ± 5.33	Infusion region x infusion timing (F(2,44) = 1.31, p = .281) Main effect of infusion region (F(2,44)= 1.09, p = .345) Main effect of infusion timing (F(1,22) = 2.10, p = .161)	53.1 ± 4.12	Infusion region x infusion timing (F(2, 44) = 1.38, p = .262) Main effect of infusion region (F(2,44) = .481, p = .621) Main effect of infusion timing (F(1,22) = 1.66, p = .211)
		HPC	90.9 ± 6.88		51.2 ± 4.13	
		mPFC	76.7 ± 6.23		47.0 ± 3.82	
	Pre-test	Vehicle	92.5 ± 6.57		52.6 ± 3.01	
		HPC	93.8 ± 4.86		57.7 ± 3.66	
		mPFC	94.0 ± 3.65		56.5 ± 4.85	
Figure 3F Propranolol	Pre-sample	Vehicle	52.4 ± 4.05	Infusion region x infusion timing (F(2,40) = .868, p = .482) Main effect of infusion region (F(2,40)= 1.63, p = .208) Main effect of infusion timing (F(1,20) = 2.38, p = .138)	34.9 ± 3.90	Infusion region x infusion timing (F(2,40) = .915, p = .409) Main effect of infusion region (F(2,40)= 1.81, p = .176) *Main effect of infusion timing (F(1,20) = 7.43, p = .013)
		HPC	54.5 ± 4.15		28.7 ± 2.98	
		mPFC	50.2 ± 4.69		27.0 ± 2.62	
	Pre-test	Vehicle	55.9 ± 5.97		38.5 ± 5.36	
		HPC	71.6 ± 8.48		40.6 ± 5.16	
		mPFC	61.6 ± 10.0		35.0 ± 5.47	
Figure 3H UK 14,304	Pre-sample	Vehicle	71.9 ± 5.65	Infusion region x infusion timing (F(2,44) = .341, p = .713) Main effect of infusion region (F(2,44) = .423, p = .658) Main effect of infusion timing (F(1,22) = 2.68, p = .116)	46.9 ± 4.07	Infusion region x infusion timing (F(1.45, 31.9) = 1.82, p = .175) Main effect of infusion region (F(1.45, 31.9) = .571, p = .517) *Main effect of infusion timing (F(1,22) = .491, p = .037)
		HPC	69.4 ± 4.97		46.9 ± 3.69	
		mPFC	65.6 ± 5.87		40.8 ± 3.89	
	Pre-test	Vehicle	59.3 ± 4.07		31.3 ± 3.72	
		HPC	60.0 ± 5.61		36.7 ± 3.77	
		mPFC	59.1 ± 3.93		36.9 ± 4.85	
Figure 3J RS79488	Pre-sample	Vehicle	69.4 ± 5.17	Infusion region x infusion timing (F(2,44) = .516, p = .600) Main effect of infusion region (F(2,44)= .026, p = .974) *Main effect of infusion timing (F(1,22) = 4.83, p = .039)	36.1 ± 3.77	Infusion region x infusion timing (F(2, 44) = .003, p = .997 Main effect of infusion region (F(2,44) = .465, p = .631) Main effect of infusion timing (F(1,22) = .499, p = .487)
		HPC	71.1 ± 4.19		38.5 ± 4.83	
		mPFC	67.3 ± 5.08		39.6 ± 3.61	
	Pre-test	Vehicle	60.4 ± 3.72		40.9 ± 3.00	
		HPC	57.2 ± 4.97		42.5 ± 2.69	
		mPFC	62.6 ± 5.51		39.4 ± 4.13	

Table 7. Analysis of performance against chance of HPC or mPFC-infused animals

Figure and drug	Infusion timing	Drug condition	Statistical analysis comparing performance against chance
Figure 3E Prazosin	Pre-sample	Vehicle	t(11) = 5.13, p < .001
		HPC	t(11) = 4.18, p = .002
		mPFC	t(11) = 4.78, p = .001
	Pre-test	Vehicle	t(11) = 4.31, p = .001
		HPC	t(11) = 3.32, p = .007
		mPFC	t(11) = 3.47, p = .005
Figure 3F Propranolol	Pre-sample	Vehicle	t(10) = 4.45, p = .001
		HPC	t(10) = -6.15, p = .553
		mPFC	t(10) = 5.10, p < .001
	Pre-test	Vehicle	t(10) = 3.82, p = .003
		HPC	t(10) = 4.40, p = .001
		mPFC	t(10) = 3.88, p = .003
Figure 3H UK 14,304	Pre-sample	Vehicle	t(11) = 4.19, p = .002
		HPC	t(11) = 1.45, p = .176
		mPFC	t(11) = 5.69, p < .001
	Pre-test	Vehicle	t(11) = 3.98, p = .002
		HPC	t(11) = 5.41, p < .001
		mPFC	t(11) = 3.20, p = .008
Figure 3J RS79488	Pre-sample	Vehicle	t(11) = 5.74, p < .001
		HPC	t(11) = 6.04, p < .001
		mPFC	t(11) = 4.34, p = .001
	Pre-test	Vehicle	t(11) = 3.26, p = .008
		HPC	t(11) = 7.13, p < .001
		mPFC	t(11) = 5.03, p < .001