Neuropsychopharmacology (2008) 33, 2831–2846

www.neuropsychopharmacology.org

Memory Processing in the Avian Hippocampus Involves Interactions between β-Adrenoceptors, Glutamate Receptors, and Metabolism

Marie E Gibbs*[,1], David N Bowser[2,4], Dana S Hutchinson[3], Richard E Loiacono[3] and Roger J Summers[3]

[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia; [2]Department of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia; [3]Department of Pharmacology, Monash University, Clayton, VIC, Australia

Noradrenaline is known to modulate memory formation in the mammalian hippocampus. We have examined how noradrenaline and selective β-adrenoceptor (AR) agonists affect memory consolidation and how antagonists inhibit memory consolidation in the avian hippocampus. Injection of selective β-AR agonists and antagonists at specific times within 30 min of a weakly or strongly reinforced, single-trial, bead discrimination learning test in 1-day-old chicks allowed us to determine the pattern of β-AR involvement in hippocampal memory processing. Different β-AR subtypes were recruited in temporal sequence after learning in the order β_1, β_3, and β_2. We provide evidence that the effect of manipulation of β_1-ARs by selective agonists and antagonists within 2.5 min of training parallels the action of NMDA receptor agonists and antagonists. Activation of β_3- and β_2-ARs facilitated memory but utilized different mechanisms: β_3-ARs by stimulating glucose uptake and metabolism, and β_2-ARs by increasing the breakdown of glycogen—with both metabolic events occurring in astrocytes and affecting intermediate memory. The different receptors are activated at different times within the lifetime of labile memory and within 30 min of learning. We have defined separate roles for the three β-ARs in memory and demonstrated that the avian hippocampus is involved in learning and memory in much the same way as the hippocampus in the mammalian brain.
Neuropsychopharmacology (2008) **33,** 2831–2846; doi:10.1038/npp.2008.5; published online 6 February 2008

Keywords: astrocytes; β-adrenoceptors; chick; discrimination-learning; hippocampus; memory

INTRODUCTION

The role of noradrenergic systems in memory storage is complex. Noradrenaline is necessary for the acquisition and consolidation of new information into long-term storage and has roles in arousal and reinforcement (Gibbs and Summers, 2002a; Berridge and Waterhouse, 2003). Noradrenergic terminals are widely distributed throughout the brain and noradrenaline acts on different adrenoceptor (AR) subtypes that potentially influence memory. Noradrenaline controls memory formation of single-trial learning in the young chick, at different times after learning and in different brain regions, including the basal ganglia (Gibbs and Summers, 2003a), locus coeruleus (Gibbs and Summers, 2003b), and multimodal intermediate medial mesopallium (IMM; Reiner, 2005) (Gibbs and Summers, 2002a, 2005).

*Correspondence: Dr ME Gibbs, Department of Anatomy and Cell Biology, Monash University, Wellington Road, Clayton, VIC 3800, Australia, Tel: +61 3 9905 2714, Fax: +61 3 9905 8192,
E-mail: marie.gibbs@med.monash.edu.au
[4]Current address: The Howard Florey and Mental Health Research Institutes, Melbourne, VIC, Australia.
Received 2 November 2007; revised 26 December 2007; accepted 2 January 2008

In the young chick, the single-trial bead discrimination task allows dissection of memory into stages that can be revealed by testing separate chicks at different times after training with or without pharmacological interventions. Memory storage is divided into three sequential stages: short-term memory (STM), intermediate memory (ITM), and long-term memory (LTM) (Mark and Watts, 1971; Watts and Mark, 1971; Gibbs and Ng, 1977; Gibbs and Summers, 2002b), which have durations that are characteristic for particular learning tasks. There are two versions of the task: one where one of two colored beads has a concentrated aversive taste producing strongly reinforced learning, and another where diluted aversant is associated with one of the two beads and produces weakly reinforced learning. Strongly reinforced learning passes through the stages of STM, ITM phases A and B (ITMA and ITMB) to LTM, is separated by brief amnestic periods, and results in good memory on testing at 120 min. Weakly reinforced learning results in a labile memory that lasts for 30 min but is not permanent. However, weakly reinforced learning can be converted to LTM by (i) repetition (Crowe *et al*, 1989), (ii) behavioral events causing arousal (Field *et al*, 2007), (iii) isolation (Johnston and Rose, 1998), or (iv) pharmacological intervention. STM lasts for 10 min in both weak and strong versions of the task, but ITM is reduced

from 55 to 30 min after weakly reinforced training. This is part of the basis for dividing ITM into ITMA and ITMB (Gibbs and Summers, 2002a).

It is thought that long-term potentiation (LTP) is the basis for memory, and many studies investigating memory focus on synaptic plasticity of LTP within the mammalian hippocampus (Izquierdo *et al*, 2006). Although the present paper does not contain electrophysiological studies, we believe that comparisons between chick and mammals are valid. In the avian hippocampus, forms of synaptic plasticity similar to those occurring in mammals have been described in avian cortical and hippocampal slices (Wieraszko and Ball, 1991; Margrie *et al*, 1998, 2000; Perkel *et al*, 2002; Ding and Perkel, 2004). Like avian or mammalian memory storage, hippocampal LTP has three phases: early-LTP (LTP-1) (Raymond and Redman, 2006; Raymond, 2007), which is short lasting and dependent on modification of key synaptic pathways; a more persistent phase or late-LTP of intermediate duration (LTP-2) requiring new protein synthesis; and finally LTP-3, which is dependent on gene transcription. The induction of late-LTP requires associative activation of heterosynaptic inputs and synergistic activation of glutamatergic and noradrenergic or dopaminergic reinforcing inputs within a specific effective time window, about 30 min after induction (Reymann and Frey, 2007). In some forms of LTP, its induction is dependent upon glutamatergic activation of *N*-methyl-D-aspartic acid receptors (NMDA-Rs), but this alone is not sufficient (Raymond and Redman, 2006; Reymann and Frey, 2007) and, in some forms of LTP, it has been shown that noradrenergic enhancement mediated by activation of β-ARs or dopamine is also necessary in mammals (Thomas *et al*, 1996; Bramham *et al*, 1997; Harley, 1998; Swanson-Park *et al*, 1999; Straube *et al*, 2003; Gelinas and Nguyen, 2005) as well as in birds (Bradley *et al*, 1995). The close relationship between LTP and NMDA-R activation is striking, and the experiments in the present paper on NMDA activation/inhibition would indirectly support a role for LTP in avian memory.

The present study investigates the role of β_1-, β_2-, and β_3-ARs in memory acquisition and consolidation in the chick hippocampus and examines the relationship with NMDA-Rs and metabolism. The neuropharmacology of memory in the chick, in bead discrimination and passive avoidance learning (Rose, 2000), has focused on the avian 'cortical' region or IMM (Gibbs and Ng, 1977; Gibbs and Summers, 2002a), but there are suggestions from lesion (Sandi *et al*, 1992) and immunohistochemical studies (Unal *et al*, 2002; Nikolakopoulou *et al*, 2006) that the hippocampus has a role in memory storage in the chick just as in mammals. The chick provides a powerful animal model to study the cellular mechanisms of memory, since the hippocampus, although lacking the characteristic laminated structure of the mammalian hippocampus, subserves similar functions in learning (Colombo and Broadbent, 2000) and, in particular, in spatial learning (Clayton and Krebs, 1994; Lee *et al*, 1998; Tommasi *et al*, 2003). The hippocampus in the bird is located conveniently on the dorsal surface of the forebrain providing easy access for injections. It is divided into similar regions to those of mammals on the basis of neurotransmitter systems (Erichsen *et al*, 1991) and connectivity with other telencephalic regions (Csillag *et al*, 1994; Szekely *et al*, 1994; Szekely, 1999; Atoji and Wild, 2006).

MATERIALS AND METHODS

Animals and Housing

Rhode Island Red × New Hampshire male chicks (35–40 g) were obtained on the morning of each experiment from a local poultry farm (Wagner's Poultry, Coldstream, VIC, Australia). The experimental conditions are described in detail elsewhere (Gibbs and Summers, 2002a). Chicks are placed in pairs on arrival at the laboratory, and each chick differentiated by their natural color variation into dark and light. The box temperature is maintained between 26 and 30°C by white 15 W pilot lamps above each cage and humidity is around 30%. Groups are made up of 16 chicks.

All experimental procedures were in accordance with the guidelines approved by the Monash University Animal Ethics Committee and comply with the 1997 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All efforts were made to minimize both the suffering and the number of animals used. Chicks were killed at the completion of each experiment by CO_2 inhalation.

Learning Paradigm

Chicks are allowed 2–3 h to become familiar with their new environment, including the presentation of beads to peck at. Initially, two 10-s presentations (30 min apart) of a small (2 mm diameter) shiny metal bead on the end of a 20-cm stiff wire were made, after which there were presentations (2.5 min apart) of red and blue glass beads (4 mm diameter) dipped in water to ensure that chicks peck at both colored beads with an equal preference for both colors prior to training. For the training trial, commencing at least 30 min later, chicks are presented with an identical red bead dipped in either 100 or 20% methyl anthranilate (Sigma-Aldrich Inc., St Louis, MO, USA) for strongly or weakly reinforced training, respectively. Chicks were allowed up to 10 s to peck but generally pecked the beads within the first 1–2 s. Memory retention, at specified intervals after training, was tested by presentation of clean, dry blue or red beads for 10 s. The discrimination ratio (DR) between red and blue beads (the number of pecks at the blue bead relative to the total number of pecks at the red and blue bead) was calculated. The pecks are recorded on a hand-held data logger and decoded by computer at the completion of the experiment. When a chick remembers the aversive taste, it avoids or gives one or two pecks at the red bead and up to 12 or more pecks at the blue bead with the DR approaching 1.0. When a chick does not remember, the DR approaches 0.5 (pecks equally at red and blue beads). Individual DRs were obtained for each chick and data are presented as mean ± SEM. Chicks that did not peck the bead during the training trial (did not train), or avoided the blue bead on test, perhaps due to generalized avoidance or nonspecific performance effects, were eliminated from the data analysis at the completion of the experiment. These exclusions resulted in the loss of only one or two chicks, leaving 14–16 chicks per group.

Drugs and Injections

The authors thank Bristol-Myers Squibb (Noble Park, VIC, Australia) for zinterol hydrochloride and Dr G Anderson (Ciba-Geigy AG, Australia) for (±)-CGP20712A. Other chemicals were (±)-ICI118551 (Imperial Chemical Industries, Wilmslow, Cheshire, England); RO363 (Institute of Drug Technology, Boronia, VIC, Australia); anisomycin, CL316243, glucose, 2-deoxyglucose (2-DG), DAB (1,4-dideoxy-1,4-imino-D-arabinitol), DNQX, SR59230A (Sigma-Aldrich Inc.); D-APV (D-(−)-2-amino-5-phosphonopentanoic acid), NMDA (Tocris Co., UK). All drugs were resuspended and diluted in sterile 0.9% physiological saline. Doses are expressed as pmol or nmol per hemisphere for central injections or per chick for subcutaneous injections (see Table 1 for doses and concentrations of drugs used).

Drugs (1 μl) were administered centrally by direct bilateral injection into the hippocampus of each hemisphere using a 50-μl repeating Hamilton syringe dispenser. Central injections were made using the tactile landmarks of the tegmentum and midline to target the injection site (1–1.5 mm from the midline, 1.0 mm from the tegmentum) (Figure 1). The depth of the injection was controlled to 1.5 mm from the surface of the head by a plastic sleeve on a 27-gauge needle. This depth included the 1.0 mm orifice of the needle, which was directed toward the back of the brain. The accuracy of placement, measured by the site of needle puncture on the skull after the animals were killed, was very high: (mean and SEM left and right of the midline) 1.21 ± 0.09 mm (left hemisphere) and 1.18 ± 0.10 mm (right hemisphere), and (from tegmentum) 1.02 ± 0.06 mm (left hemisphere) and 1.05 ± 0.06 mm (right hemisphere) ($n = 28$). For subcutaneous administrations, 100 μl was injected into a fold of skin on the ventral side of the thorax. To demonstrate injection sites in the hippocampus, we injected chicks with FM1–43 (1 μg/μl; Invitrogen, Mount Waverley, VIC, Australia). This lipophilic dye rapidly inserts into the outer leaflet of cell membranes. Once injected, chicks were decapitated, brains rapidly removed and frozen, and 30 μm coronal cryosections collected sequentially through the hippocampal region. Sections were mounted on microscope slides and imaged with an Andor 885 EMCCD camera attached to an inverted Olympus IX Microscope with FITC fluorescence filters (excitation 490BP30 and emission 515LP). A series of low-power images were collected and a montage was created using Adobe Photoshop CS3.

Reverse Transcription-Polymerase Chain Reaction

One-day-old chicks were killed by decapitation, brains removed, and the IMM or hippocampus dissected from other brain regions. Brain regions were rapidly frozen in liquid nitrogen and stored at −80°C until use, and RNA was extracted using Trizol (Invitrogen). The yield and quality of RNA was assessed by measurement of absorbance at 260 and 280 nm and electrophoresis on 1.0% agarose gel. cDNAs were synthesized by reverse transcription of 1 μg of each total RNA using oligo(dT_{15}), and polymerase chain reaction (PCR) was performed using primers specific for chick β_1-AR, β_2-AR, or β_3-AR (Hutchinson *et al*, 2007). Following amplification, PCR products were electrophoresed on 1% agarose gels and images captured digitally.

Autoradiography

Brains were removed and frozen slowly over liquid nitrogen by placing the brain on foil on a cork floating in liquid nitrogen. The brain cools quickly and freezes without distortion. Sections (10 μm) were cut on a Cryostat at −20°C and mounted onto gelatin-coated microscope slides. Slide-mounted sections were preincubated at 25°C for 30 min in Krebs buffer (composition (in mM): NaCl 118.4, KCl 4.7, $MgSO_4.7H_2O$ 1.2, $NaH_2PO_4.2H_2O$ 10, $CaCl_2$ 1.27) containing 0.1 mM ascorbic acid and 10 μM phenylmethylsulfonylfluoride with 0.1 mM GTP, and then 2.5 h in Krebs buffer with [^{125}I]cyanopindolol (50 pM) at 25°C with or without ICI118551 (70 nM) or CGP20712A (100 nM) to define β_1- and β_2-ARs, respectively. Nonspecific binding was determined with (−)-propranolol (1 μM). Labeled sections were rinsed quickly in buffer followed by 2 × 15 min washes at 37°C in buffer and finally rinsed in distilled water (25°C). Sections were then dried with a cold stream of nitrogen and acetone vapor. Slides were exposed to X-ray film for 48 h before development of films.

Experimental Design

Dose–response relationships. Dose–response curves were constructed for drugs injected either into the hippocampus or subcutaneously, depending on the particular experiment. In some cases, doses used were based on our published experiments where drugs were administered into the IMM.

The ability of agonists to enhance memory was revealed using weakly reinforced training (20% anthranilate) and the

Table 1 Selective Noradrenergic Subtype Agonists and Antagonists and Doses (Concentration Used for 1 μl Injections into IMM or 100 μl Subcutaneous Injections)

Receptor	Agonist	Optimal dose pmol/hemisphere (concentration)	Antagonist	Optimal dose pmol/hemisphere (concentration)	Subcut. dose, pmol/chick (concentration)
β_1-AR	RO363	3–10 (3–10 μM)	CGP20712A	2 (2 μM)	1 (0.01 μM)
β_2-AR	Zinterol	30 (30 μM)	ICI118551	10 (10 μM)	
β_3-AR	CL316243	3 (3 μM)	SR59230A	100 (100 μM)	
NMDA-R	NMDA	1 nmol (1 mM)	D-APV	30 (30 μM)	1 (0.01 μM)

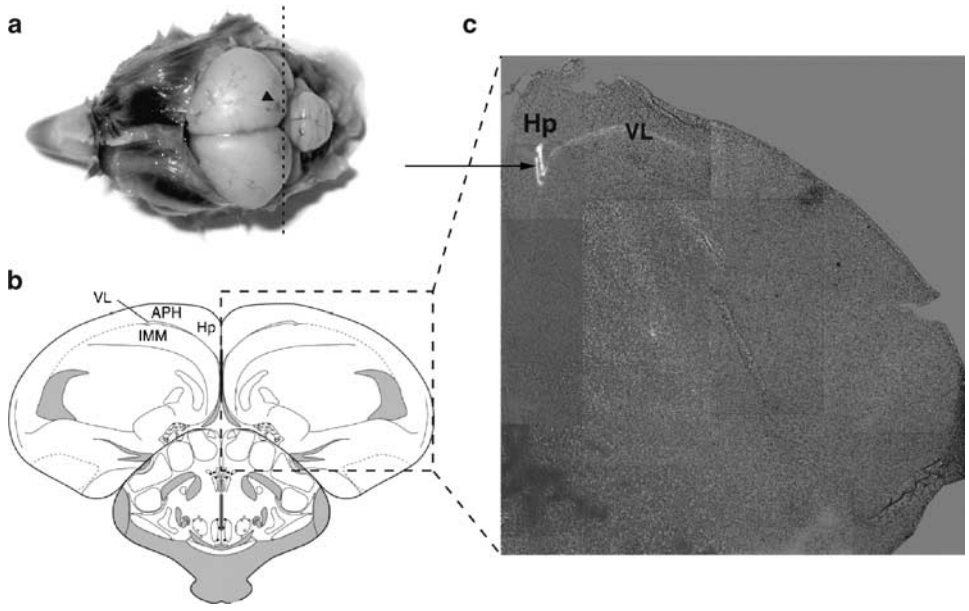

Figure 1 Illustration of the point of injection into the chick brain. (a) Image of a chick head with the skull removed. Injection site on right hemisphere indicated by arrowhead. Dotted line indicates level of coronal section presented in panels b and c. (b) Cartoon adapted from the images of Dr Wayne Kuenzel (Plate 7.8; http://avianbrain.org/nomen/Chicken_Atlas.html) with box indicating the region of interest shown in panel c. (c) Montage of fluorescence images taken at low power, indicating the site of FM1–43 injection into the right hippocampal area. Arrow showing needle track. VL, ventricle; Hp, hippocampus; IMM, intermediate medial mesopallium.

ability of antagonists to inhibit was revealed using strongly reinforced training (100% anthranilate). Low doses of antagonists were used to reduce the ability of an agonist to enhance labile memory after weakly reinforced training. Memory, unless otherwise indicated, was tested 120 min after training (after LTM had been established).

Time of injection and test. Injections, using dose levels established from earlier IMM experiments, were made in separate groups of chicks at times between 5 min before and up to 40 min after training to determine the times at which the drugs enhanced or impaired memory consolidation. With a time of injection selected on the basis of this, a number of groups of chicks were injected with the inhibitor, and memory retention was tested in separate groups of chicks at discrete time intervals after training. In this way, the timing of the memory loss and the stage of memory affected by the drug were determined.

Specificity of drug–receptor interactions. To determine selectivity of drugs to particular receptors, or particular cellular mechanisms, the memory-enhancing properties of agonists were challenged by prior administration of a fixed dose of selective antagonist or inhibitor injected subcutaneously 5 min before the agonist. β-AR receptor specificity was determined with selective agonists and antagonists with comparisons made between β_1-AR activity and effects of NMDA-Rs, and β_2- and β_3-AR activity and the effects of metabolic inhibitors of glycogenolysis (DAB), protein synthesis (anisomycin), and glycolysis (2-DG). Facilitation of adrenergic memory enhancement was employed to show if enhancement was increased in the presence of glucose.

Data Analysis

The results for each experiment were analyzed using SPSS (Information Analysis Systems SPSS Inc., Chicago, IL, USA) with one- and two-way independent measures ANOVA with either Dunnett's *t*-test or simple main effect *post hoc* analyses, where appropriate. Although sample sizes differed, this was not due to experimental factors; therefore, all analyses used unweighted means. Two-tailed tests of significance were conducted and a type I error rate of 0.05 was adopted.

RESULTS

Strongly and Weakly Reinforced Memory Paradigms

Injection of saline into the hippocampus had no effect on memory processing for either strongly (100% anthranilate) or weakly reinforced training (20% anthranilate). Testing 120 min after training showed that chicks trained on 100% anthranilate had strong memory with all saline injections between −5 and +30 min, whereas chicks trained on 20% anthranilate had no memory with any injection (Figure 2a) ($F_{(5,169)} = 0.83$; $p < 0.001$). The DRs from weakly and strongly reinforced training were significantly different ($F_{(1,169)} = 122.32$; $p < 0.001$). When chicks were injected with saline 2.5 min after training on 20% anthranilate and tested between 10 and 120 min, the chicks showed high DRs and strong memory 10 and 30 min post-training, but low DRs at 60 and 120 min ($F_{(1,91)} = 16.33$ and 22.51, respectively, $p < 0.001$). This is indicative of a labile memory period with weakly reinforced training that does not consolidate beyond 30 min (Figure 2b). Retention levels in

control chicks injected with saline are not significantly different from those seen in experiments where there were no injections (Gibbs and Ng, 1979).

Role of Hippocampal β_1-ARs in Memory Formation

Receptor autoradiography conducted under conditions that would allow identification of β_1- and β_2-ARs demonstrated the presence of both receptors in the chick mesopallium and hippocampus with the binding levels of ICYP to β_2-ARs being higher than those for β_1-ARs in both regions (Figure 3a). These results are in agreement with previous studies in the avian brain, where ICYP binding attributable to β_2-ARs is higher in the chick and pigeon mesopallium and hippocampus as compared to β_1-AR binding (Fernandez-Lopez *et al*, 1997). However, in mammalian species (baboon, rat, guinea-pig), β_1-AR binding was higher in all brain regions, apart from the hippocampus, where β_2-AR binding was higher in baboons (Slesinger *et al*, 1988) but similar levels in rat and guinea-pig hippocampus (Booze *et al*, 1989). Another study in rat hippocampus showed higher β_1-AR populations as compared to β_2-ARs (Rainbow *et al*, 1984). The results from the autoradiography experiments in this study correlate well with the RT-PCR for mRNA in these regions (β_3-AR mRNA is also detected in these regions) (Figure 3b), and that found in mammalian studies where β_1-, β_2-, and β_3-AR mRNA has been detected (Nicholas *et al*, 1996; Summers *et al*, 1995).

Injections of 1 μl of the selective β_1-AR agonist RO363 (3 pmol/hemisphere) into the hippocampus promoted memory for weakly reinforced training, provided the agonist was injected within 2.5 min of training (Figure 4a; $F_{(7,120)} = 7.932$; $p < 0.001$). Injection for 5 min or more after training failed to promote memory formation (see

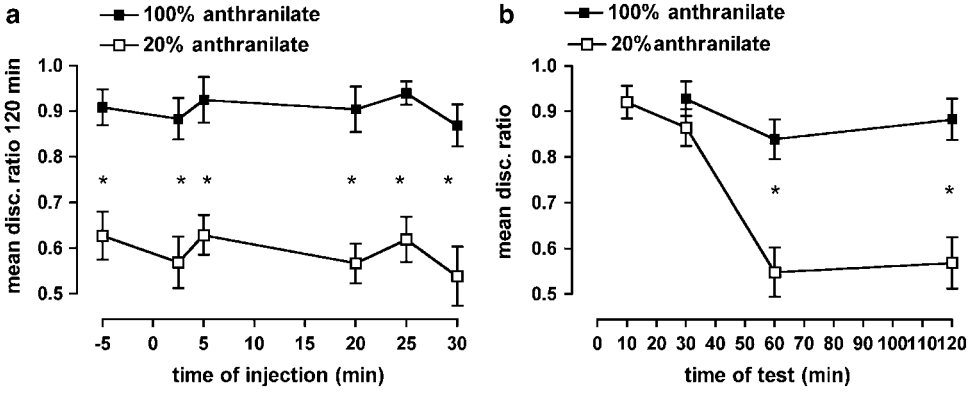

Figure 2 Memory retention following weakly and strongly reinforced training (20 and 100% anthranilate). (a) Chicks given hippocampal injections of saline at times between 5 min before and 30 min after training and tested at 120 min. (b) Injections of saline at 2.5 min and chicks tested between 10 and 120 min after weakly and strongly reinforced training. *$p < 0.05$, $N = 13$–18 per data point.

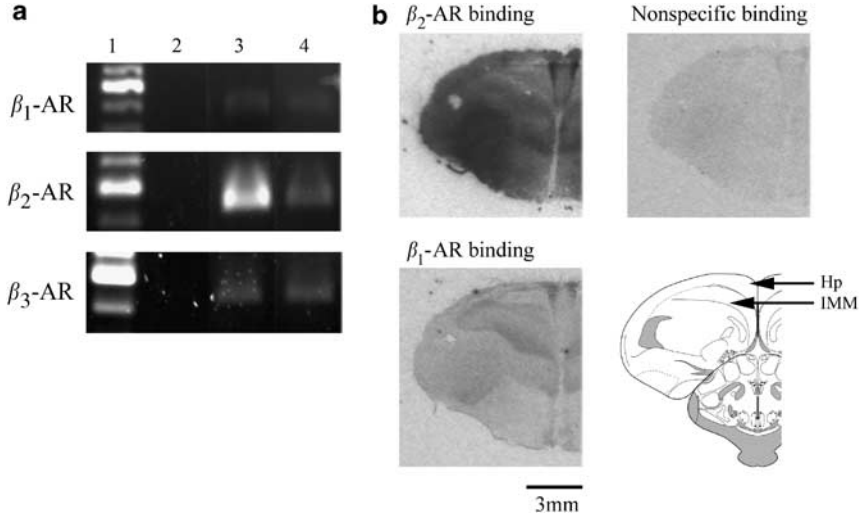

Figure 3 (a) Analysis of β_1-AR (414 bp), β_2-AR (441 bp), and β_3-AR (430 bp) PCR products showing the distribution of β-AR subtypes in the IMM and Hp in 1-day-old chick. Lane 1: 100 bp molecular weight marker (bright band is 500 bp); lane 2: negative reverse transcriptase control; lane 3: Hp; lane 4: IMM. (b) Images from X-ray film showing the distribution of [^{125}I]cyanopindolol binding sites in coronal sections of 1-day-old chick forebrain. Sections were incubated with [^{125}I]cyanopindolol (50 pM) in the presence of 100 nM CGP20712A to define β_2-AR binding, or in the presence of 70 nM ICI118551 to define β_1-AR binding, or 1 μM propranolol to define nonspecific binding. Cartoon adapted from the images of Dr Wayne Kuenzel (Plate 7.0; http://avianbrain.org/nomen/Chicken_Atlas.html).

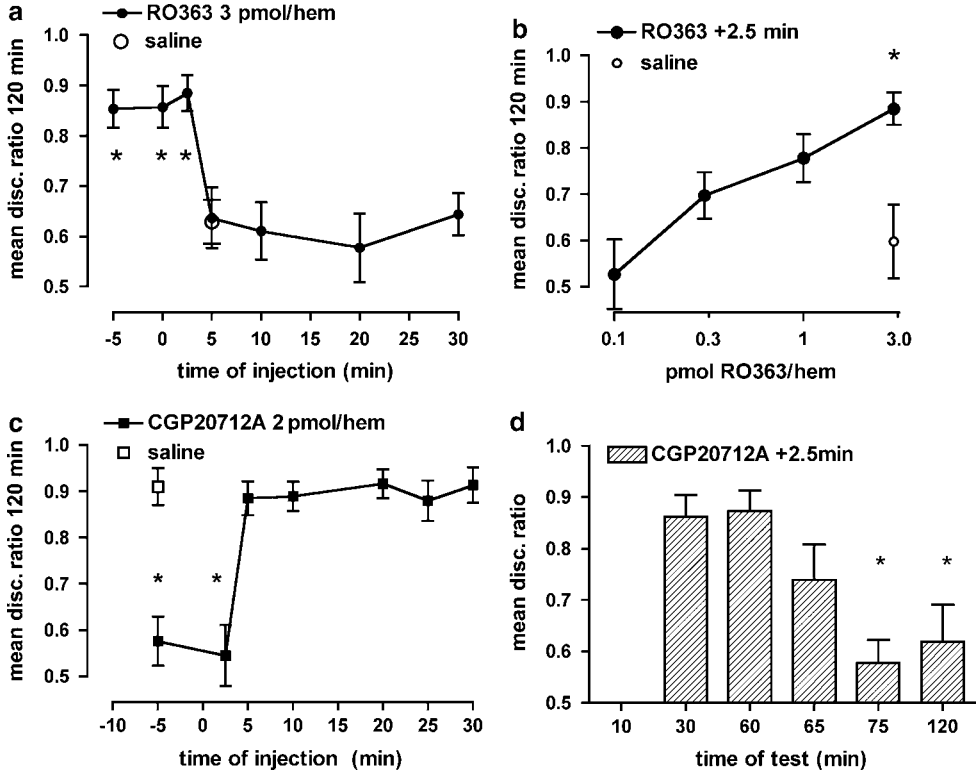

Figure 4 Effect of the selective β_1-AR agonist (RO363) and antagonist (CGP20712A) injected bilaterally into the hippocampus. (a) Discrimination ratios at 120 min following injection of RO363 (3 pmol/hemisphere) between −5 and 30 min after weakly reinforced training. Memory consolidation occurred only in chicks injected between −5 and 2.5 min relative to training. (b) Dose–response relationship for promotion of weakly reinforced training by RO363 injected 2.5 min after training. (c) Susceptibility of strongly reinforced memory to CGP20712A (2 pmol/hemisphere) between −5 and +2.5 min relative to training. Dose established from earlier experiments with injections into the IMM (Gibbs and Summers, 2005). (d) Memory loss following CGP20712A injected 2.5 min after strongly reinforced training occurred after 60 min. *$p<0.05$, $N=13$–18 per data point.

Figure 1a). Memory-enhancing effects of RO363 injected 2.5 min after training were dose-dependent (Figure 4b; $F_{(4,69)}=6.608$; $p<0.001$).

Bilateral injections of the selective β_1-AR antagonist CGP20712A (Figure 4c) into the hippocampus at a dose based on earlier work (2 pmol/hemisphere; see Gibbs and Summers, 2005) prevented memory formation and caused memory loss 120 min after strongly reinforced (100% anthranilate) training, provided that the antagonist was injected between −5 and +2.5 min of training ($F_{(7,123)}=12.37$; $p<0.001$). When CGP20712A was administered 2.5 min after training, memory was unimpaired when tested at 30 and 60 min but was clearly lost by 75 min (Figure 4d; $F_{(4,73)}=3.68$; $p<0.01$). The finding that the drug was no longer effective 5 min after training suggests that it cannot be having an effect on retrieval.

Role of Hippocampal β_2-ARs in Memory Formation

After weakly reinforced training, the β_2-AR agonist zinterol (30 pmol/hemisphere) enhanced memory when injected into the hippocampus 15–30 min after training (Figure 5a; $F_{(9,143)}=9.17$; $p<0.001$) but not earlier. Examination of the dose–response relationship at 20 min confirmed that the dose used was optimal ($F_{(3,54)}=6.67$; $p=0.001$) (Figure 5b). In contrast to the β_1-AR antagonist, the selective β_2-AR antagonist, ICI118551A (10 pmol/hemisphere), did not inhibit memory formation when injected 2.5 min after training, but was effective at 25 min (Figure 5c; $F_{(6,105)}=6.15$; $p<0.001$compared to −5 min), a time when the β_1-AR antagonist had no effect. Examination of the dose–response relationship for the β_2-AR antagonist ICI118551 indicated that an inhibitory dose similar to that found to be effective in IMM (3 and 10 pmol/hemisphere) (Figure 5d; $F_{(4,69)}=4.69$; $p=0.002$) was optimal. As with the β_1-AR antagonist, loss of memory following inhibition of β_2-ARs occurred 60–75 min after training (Figure 5e; $F_{(3,57)}=8.22$; $p<0.001$).

Role of Hippocampal β_3-ARs in Memory Formation

β_3-ARs are involved in memory processing in the IMM, and we show here that they also play a role in memory formation in the hippocampus. The selective β_3-AR agonist CL316243 (3 pmol/hemisphere) significantly enhanced memory for weakly reinforced training at all times up to 30 min ($F_{(5,90)}=6.64$; $p<0.001$) but not at 40 min (Figure 6a). The facilitation of memory by CL31643 injected 20 min after training was dose-dependent ($F_{(3,55)}=11.83$; $p<0.001$) (see Figure 6b). The β_3-AR antagonist SR59230A (100 pmol/hemisphere; Figure 6c) significantly inhibited memory when injected 2.5 or 5 min after strongly reinforced training ($F_{(6,109)}=14.69$; $p<0.001$). The effect of SR59230A injected 2.5 min after training was dose-dependent (Figure 6d; $F_{(3,59)}=8.71$; $p<0.001$). Inhibition of memory was gradual following injection at +2.5 min, being significant at 60 and 120 min after training (Figure 6e; $F_{(3,55)}=5.07$; $p=0.004$).

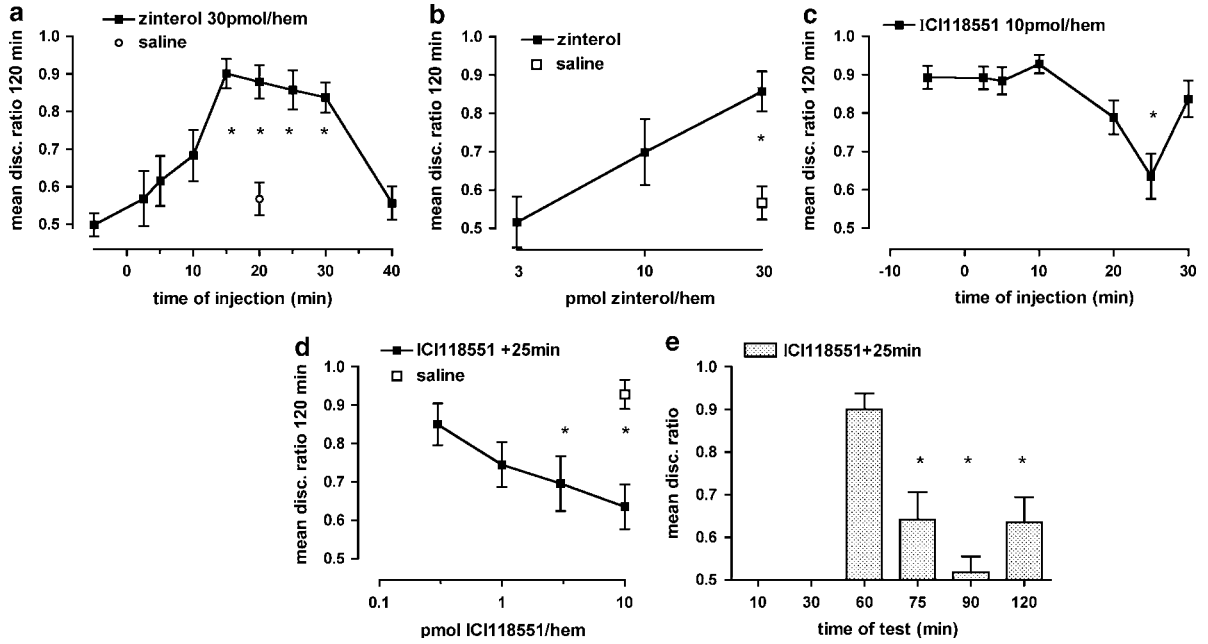

Figure 5 Effect of β_2-AR agonist (zinterol) and antagonist (ICI118551) on memory storage in the hippocampus. (a) Memory 120 min after injection of zinterol (30 pmol/hemisphere) at different times after weakly reinforced training. (b) Dose–response relationship for the β_2-AR agonist zinterol injected 20 min after weakly reinforced training. (c) Time of administration of ICI118551 (10 pmol/hemisphere) to determine effective inhibitory times of administration. (d) Dose–response relationship for ICI118551 injected 25 min after strongly reinforced training. (e) Chicks injected with ICI118551 25 min after training were tested at selected times after training. $^*p<0.05$, $N=14$–16 per data point.

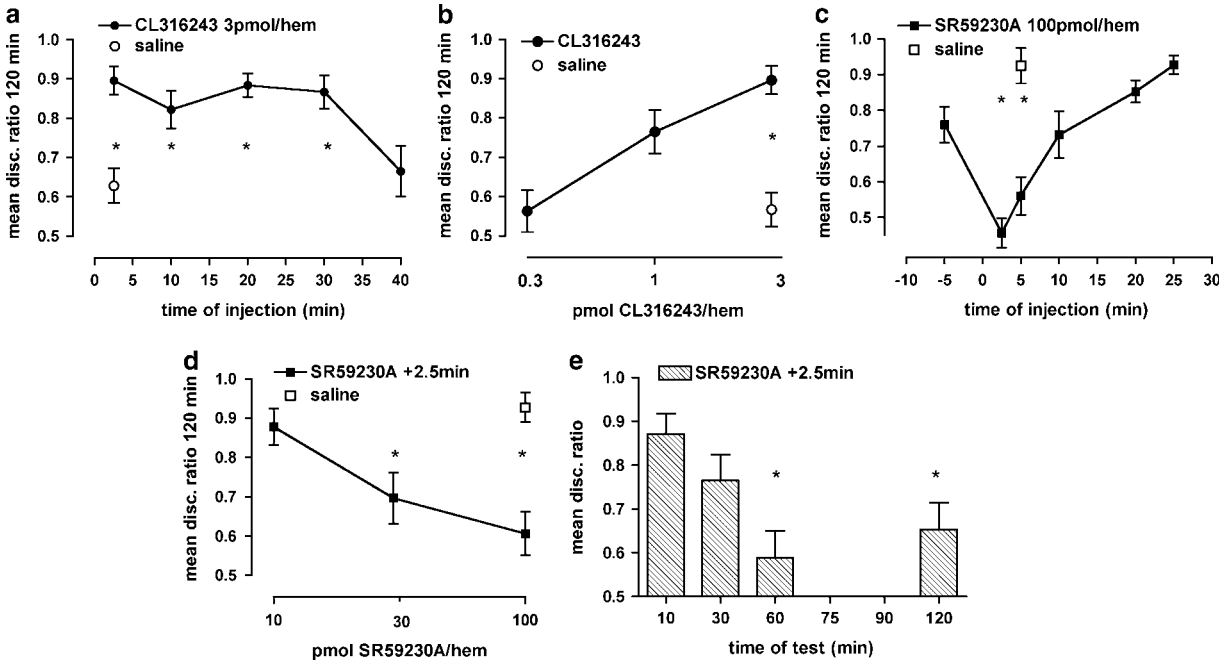

Figure 6 Effect of β_3-AR agonist (CL316243) and antagonist (SR59230A) on memory storage in the hippocampus. (a) Memory 120 min after injection of the β_3-AR agonist CL316243 (3 pmol/hemisphere) at different times after weakly reinforced training. (b) Dose–response relationship for CL36243 injected 20 min after weakly reinforced training. (c) Time of administration of the β_3-AR antagonist S59230A (100 pmol/hemisphere) to determine effective inhibitory times of administration. (d) Dose–response relationship for SR59230A injected 2.5 min after strongly reinforced training. (e) Memory loss following SR59230A injected 2.5 min after strongly reinforced training commenced after 30 min, and was significant by 60 min. $^*p<0.05$, $N=14$–16 per data point.

Selectivity of β-AR Agonists and Antagonists in Memory Processing at Hippocampal β-ARs

To verify the selectivity of the β-AR agonists, we constructed dose–response curves to the agonists in the presence of low doses of selective antagonists. For β_1-AR agonists, subcutaneous injection of the antagonists at a dose that had no significant effect on memory was made 5 min before training, with RO363 injected at +2.5 min. The dose–response relationships for β_2- and β_3-AR agonists

injected into the hippocampus 20 min after weakly reinforced training were challenged by subcutaneous preadministration (5 min) of selective β_2- and β_3-AR antagonists.

The dose–response curve to the selective β_1-AR agonist RO363 was shifted to the right by preadministration of the β_1-AR antagonist CGP20712A (Figure 7a; 1 pmol/chick; dose effect $F_{(2,88)} = 8.70$, $p < 0.001$, and drug effect comparing preadministration of saline with CGP20712A $F_{(1,88)} = 9.35$, $p = 0.003$). This subcutaneous dose of CGP20712A does not significantly inhibit memory when given alone (Gibbs and Summers, 2005).

Selectivity of activation of β_2-ARs was demonstrated by the rightward shift of the zinterol dose–response curve (Figure 7b) by the β_2-AR antagonist ICI118551 but not by the β_3-AR antagonist SR59230A (drug effect between treatments $F_{(2,85)} = 7.45$, $p = 0.001$, dose effect $F_{(1,85)} = 9.03$, $p = 0.003$). In contrast, the dose–response curve to CL316243 (Figure 7c) was shifted significantly to the right by SR59230A but not by ICI118551 (drug effect between treatments $F_{(2,90)} = 7.25$, $p = 0.001$, dose effect $F_{(1,90)} = 11.48$, $p = 0.001$). The doses of ICI118551 and SR59230A used do not directly inhibit memory (Gibbs and Summers, 2005).

Involvement of Hippocampal NMDA-Rs in Memory Consolidation

Consolidation of memory was vulnerable to modulation of NMDA-Rs. NMDA (1 nmol/hemisphere) promoted the consolidation of weakly reinforced memory when injected between −5 and +2.5 min or at 30 min relative to training (Figure 8a) ($F_{(8,132)} = 6.72$; $p < 0.001$). The effect of NMDA was dose-dependent (Figure 8b; $F_{(5,88)} = 13.47$; $p < 0.001$). Bilateral injection of the NMDA-R antagonist D-APV (30 pmol/hemisphere) at −5 to +2.5 min or at 30 min resulted in an inhibition of memory consolidation (Figure 8c; $F_{(8,131)} = 5.79$; $p < 0.001$). The time when memory was vulnerable to enhancement matched its time of vulnerability to inhibition.

Injection of D-APV (30 pmol/hemisphere) 2.5 min after strongly reinforced training resulted in a dose-dependent inhibition of memory consolidation (Figure 8d; $F_{(5,87)} = 4.40$; $p = 0.001$) tested between 60 and 120 min (Figure 8e; $F_{(3,58)} = 10.15$; $p < 0.001$).

Relationship between Hippocampal β_1-ARs and NMDA-Rs in Memory Processing

Because of the similarity of timing between the response to β_1-AR and NMDA-R activation, and the known relationship of β-ARs and LTP (see Discussion), we sought to determine if there was any functional relationship between these two receptor subtypes. We challenged the ability of the β_1-AR agonist to facilitate memory in the presence of the NMDA antagonist (D-APV) and the ability of the NMDA-R agonist to facilitate memory in the presence of the β_1-AR antagonist.

The dose of the antagonist required to challenge facilitation of memory consolidation by NMDA and the β_1-AR agonist RO363 was chosen from studies of the subcutaneous dose–response relationship of selective antagonists (Gibbs and Summers, 2005; Figure 9a; $F_{(3,58)} = 3.97$; $p = 0.012$). The β_1-AR antagonist CGP20712A (1.0 pmol/chick) and the NMDA-R antagonist D-APV (1 pmol/chick) were given 5 min prior to strongly reinforced training. These doses did not significantly inhibit memory consolidation.

The low dose of the NMDA-R antagonist given subcutaneously 5 min before weakly reinforced training caused a rightward shift in the dose–response relationship to NMDA injected (+2.5 min after training) into the hippocampus (Figure 9b) ($F_{(1,87)} = 20.11$; $p < 0.001$). Likewise, the low dose of the β_1-AR antagonist (Figure 9c) caused a rightward shift in the dose–response relationship to RO363. However, challenge of NMDA by the β_1-AR antagonist ($F_{(1,89)} = 15.86$; $p < 0.001$) or RO363 by D-APV ($F_{(1,88)} = 16.09$; $p < 0.001$) also shifted the respective dose–response curves to the right.

A low dose of the NMDA-R antagonist, that did not produce significant memory loss on its own, blocked the facilitation of memory by the β_1-AR agonist. These findings show that both the β_1-AR and the NMDA-R agonists and antagonists affect memory with the same temporal pattern

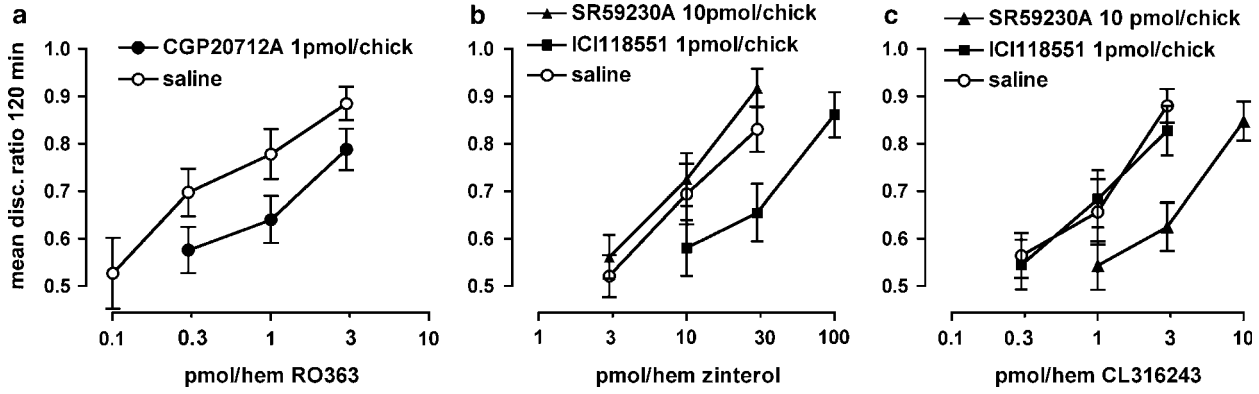

Figure 7 Selectivity of effect of β-AR agonists on their respective receptors. (a) The dose–response relationship of the β_1-AR agonist RO363 in the presence of a low dose of the selective β_1-AR antagonist CGP20712A. (b) The dose–response of the β_2-AR agonist zinterol in the presence of a low dose of the selective β_2-AR antagonist ICI118551 or the selective β_3-AR antagonist SR59230A. (c) The dose–response to the β_3-AR agonist CL316243 in the presence of a low dose of SR59230A or ICI118551. $N = 14$–16 per data point.

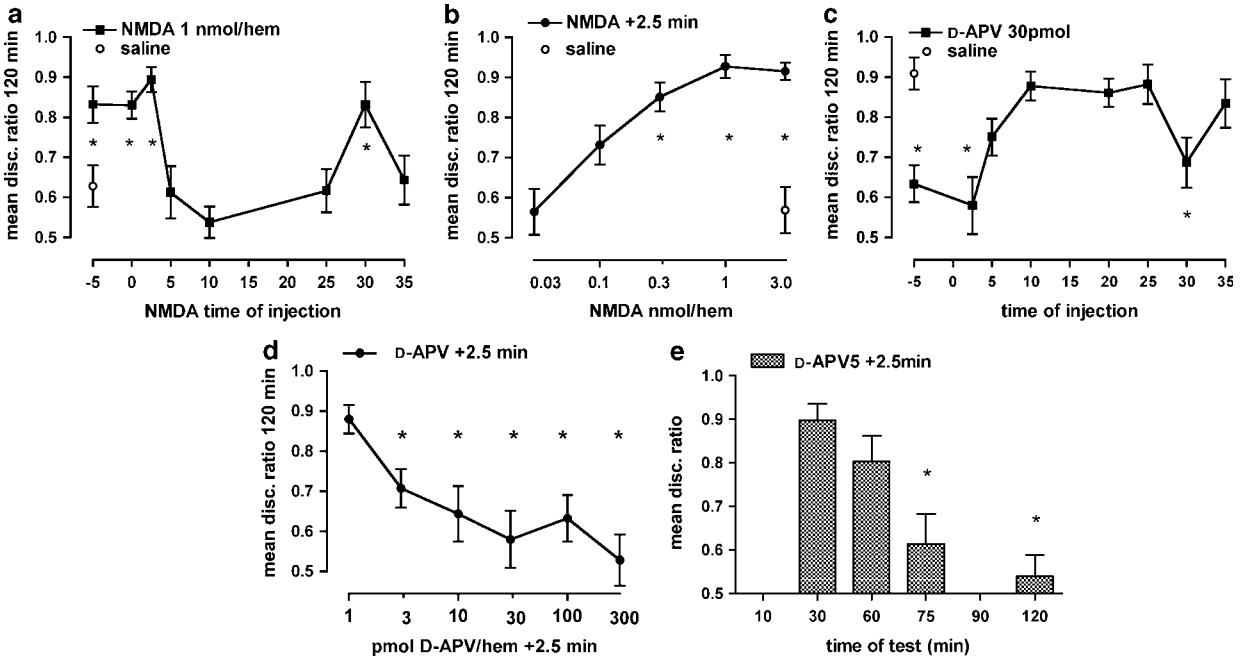

Figure 8 Effect of hippocampal administration of the NMDA-R antagonist D-APV and the agonist NMDA on memory. (a) Time relative to training when injection of NMDA led to memory enhancement, from −5 to +2.5 min. (b) Dose–response to NMDA 2.5 min after weakly reinforced training. (c) Time window between −5 and +2.5 min where injection of D-APV led to inhibition of memory tested at 120 min. (d) Dose–response to D-APV 2.5 min after strongly reinforced training. (e) Time of test following 30 pmol D-APV 2.5 min after training. *$p<0.05$, $N=14$–16 per data point.

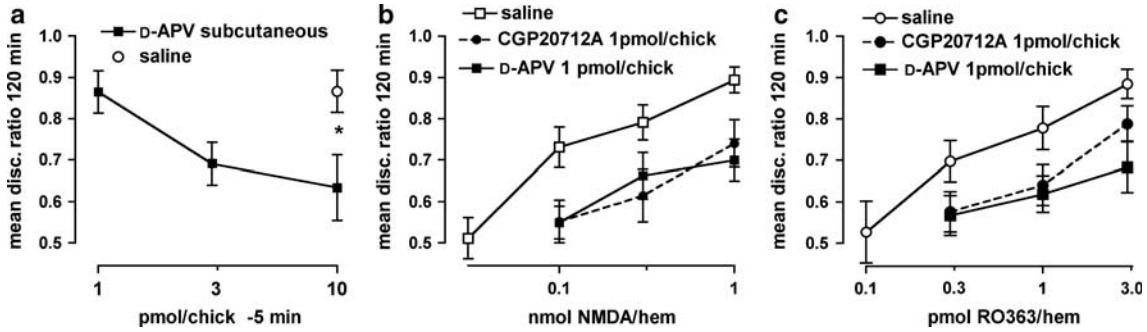

Figure 9 Receptor selectivity and the relationship between β_1-ARs and NMDA-Rs. (a) Dose–response for subcutaneous D-APV. (b) Selectivity of the NMDA-R antagonist D-APV and the β_1-AR antagonist CGP20712A against NMDA demonstrated by the rightward shift in the presence of low doses of the antagonists. (c) Dose–response to RO363 was shifted to the right by D-APV and CGP20712A (compared with the receptor antagonist shown in Figure 6a, dotted line). The challenging drugs were administered subcutaneously 5 min before and the agonists were injected into the hippocampus 2.5 min after weakly reinforced training. $N=14$–16 per data point.

and suggest that they influence memory in a related manner. The challenge experiments suggest that noradrenergic stimulation of β_1-ARs and glutamatergic stimulation of NMDA-R are both necessary for memory processing and may represent the heterosynaptic facilitation required for some forms of LTP induction.

Role of Hippocampal Protein Synthesis in Memory Formation

β-ARs are involved in the persistence or maintenance of protein synthesis-dependent LTP at a stage later than the induction phase (Swanson-Park *et al*, 1999; Straube and Frey, 2003; Straube *et al*, 2003). The protein synthesis inhibitor anisomycin injected into the hippocampus caused memory loss when injected 15 and 20 min after strongly reinforced training (Figure 10a; $F_{(6,97)}=5.86$; $p<0.001$). The effect with injection 20 min after training was dose-dependent (Figure 10b; $F_{(3,58)}=12.24$; $p<0.001$), with significant memory loss at 120 min (Figure 10c; $F_{(2,42)}=4.00$; $p=0.026$). The decrease in memory at 60 min suggests that the loss occurs between 60 and 120 min. The pattern was similar to that seen with β_2-AR antagonists injected into the hippocampus, in that memory is vulnerable just prior to consolidation at 30 min. In the hippocampus, the time during which memory is vulnerable to inhibition is much more limited (15–20 min) compared to IMM (−5 to +20 min), but the timing of memory loss also occurs after 30 min (Gibbs and Summers, 2002a).

The Role of Glucose and Glycogen in Memory Facilitation by β_2- and β_3-AR Agonists

The effects of the metabolic inhibitors, 2-DG and DAB, that inhibit memory and glucose that facilitates memory when administered into the IMM (Gibbs and Summers, 2002b; Gibbs *et al*, 2006a, 2007) were examined in the hippocampus to compare the temporal parameters in the two brain areas.

Glucose (10 nmol/hemisphere) administered up to 15 min after weakly reinforced training facilitated memory consolidation ($F_{(7,107)} = 8.74$; $p < 0.001$) (Figure 11a). Inhibition of glycolysis by 2-DG (25 nmol/hemisphere) prevented memory consolidation when injected both 5 min before and 5 min after strongly reinforced training (2-DG, 25 nmol/hemisphere ($F_{(7,118)} = 7.36$; $p < 0.001$); see Figure 11b); 2-DG also inhibited memory when injected 40 min after training. The doses of 2-DG and glucose were those established in earlier work (Gibbs and Summers, 2002b).

Inhibition of memory consolidation by the glycogenolysis inhibitor DAB (100 pmol/hemisphere) occurred after injection 5 min before or up to 25 min after strongly reinforced training ($F_{(7,118)} = 8.09$; $p < 0.001$). (Figure 11c). The dose–response curve confirmed that this was the optimal dose of

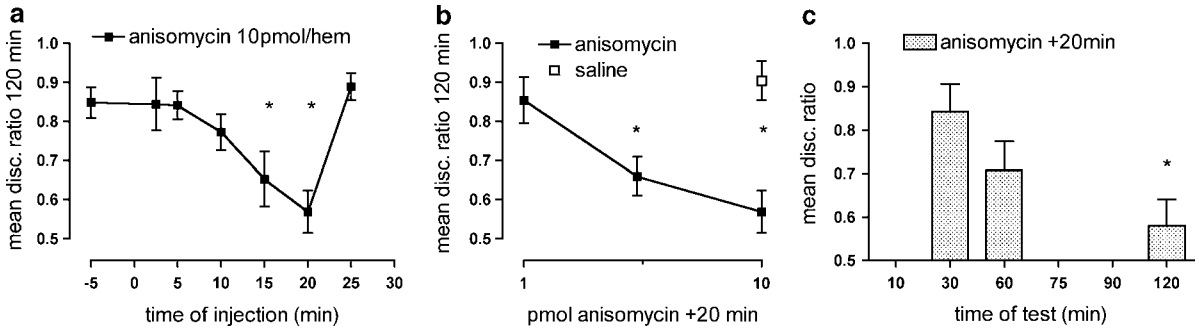

Figure 10 Effect of hippocampal injection of the protein synthesis inhibitor anisomycin. (a) Time of vulnerability of memory to hippocampal injection of anisomycin (10 pmol/hemisphere). (b) Dose–response relationship to hippocampal anisomycin injected 20 min after training. (c) Timing of loss of memory following injection 20 min after strongly reinforced memory. *$p < 0.05$, $N = 14$–16 per data point.

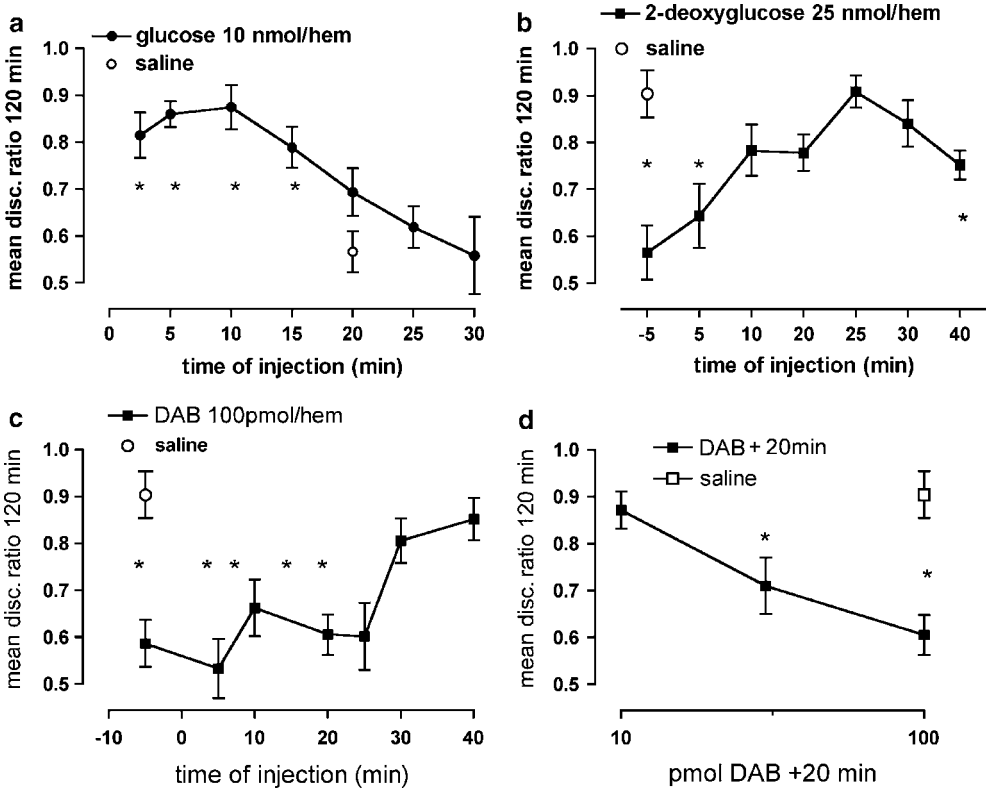

Figure 11 Effect of hippocampal injection of blockade of glycolysis (2-DG) and glycogenolysis (DAB) on strongly reinforced training. (a) Glucose enhanced weakly reinforced training with hippocampal injections up to 15 min after training. (b) 2-DG inhibited memory when administered between 5 min before and 5 min after training and also at + 40 min. (c) Hippocampal DAB was effective in inhibition of memory injected up to 25 min after training but not at 30 min or later. (d) Dose–response relationship for DAB injected into the hippocampus 20 min after training. *$p < 0.05$, $N = 12$–16 per data point.

DAB for memory inhibition ($F_{(3,59)} = 11.19$; $p < 0.001$) (Figure 11d).

Functional challenges were carried out with injections of the β-AR agonists injected into the hippocampus to determine if the action of the β_1-, β_2-, or β_3-AR agonists was related to these metabolic pathways. A low, non-facilitating, subcutaneous dose of glucose (0.3 μmol/chick s.c.) administered 5 min prior to hippocampal injection of the β_3-AR agonist (1.0 pmol/hemisphere; Figure 12b) increased the response to CL316243 and conversely a low dose of 2-DG (1 μmol/chick s.c.) decreased the response to CL316243 (Figure 12b; $F_{(5,90)} = 4.74$; $p < 0.001$). Reducing the breakdown of glycogen with DAB (0.1 nmol/chick s.c.) did not affect the ability of CL316243 to enhance memory.

In contrast, zinterol did not facilitate memory in the presence of a low dose of glucose (zinterol 10 pmol/hemisphere; Figure 12d), nor did 2-DG impair the ability of zinterol to facilitate memory, but the response to zinterol was impaired in the presence of DAB (Figure 12d; $F_{(5,86)} = 5.73$; $p < 0.001$) . The doses for glucose and 2-DG were selected from previous work (Gibbs and Summers, 2002b; Gibbs *et al*, 2007), and DAB was used at 0.1 nmol/chick s.c. (Figure 12a). A dose of DAB of 0.3 nmol/chick was required to significantly impair memory (Figure 12a) (DAB, $F_{(3,52)} = 11.54$; $p < 0.001$).

Therefore, the β_3-AR agonist acted by stimulating glucose uptake and glycolysis, with glucose enhancing the effect of CL316243, whereas 2-DG reduced the ability of CL316243 to enhance memory. In contrast, the memory-enhancing action of zinterol suggested an action via glycogenolysis, since it was reduced by DAB but was not reduced by either glucose or 2-DG.

Neither 2-DG nor DAB affected the action of the β_1-AR agonist RO363 (Figure 12c). Drugs were injected subcutaneously 5 min prior to training, and RO363 was injected into the hippocampus 2.5 min after weakly reinforced training. The actions of β_2- and β_3-AR agonists are in accord with studies involving injection into IMM. Both receptor subtypes are present on chick astrocytes (Gibbs *et al*, 2007; Hutchinson *et al*, 2007).

Comparison of β-AR Involvement in Hippocampus with Medial Striatum and IMM

β_1-ARs in hippocampus and medial striatum. The time window for administration of the β_1-AR agonist and the

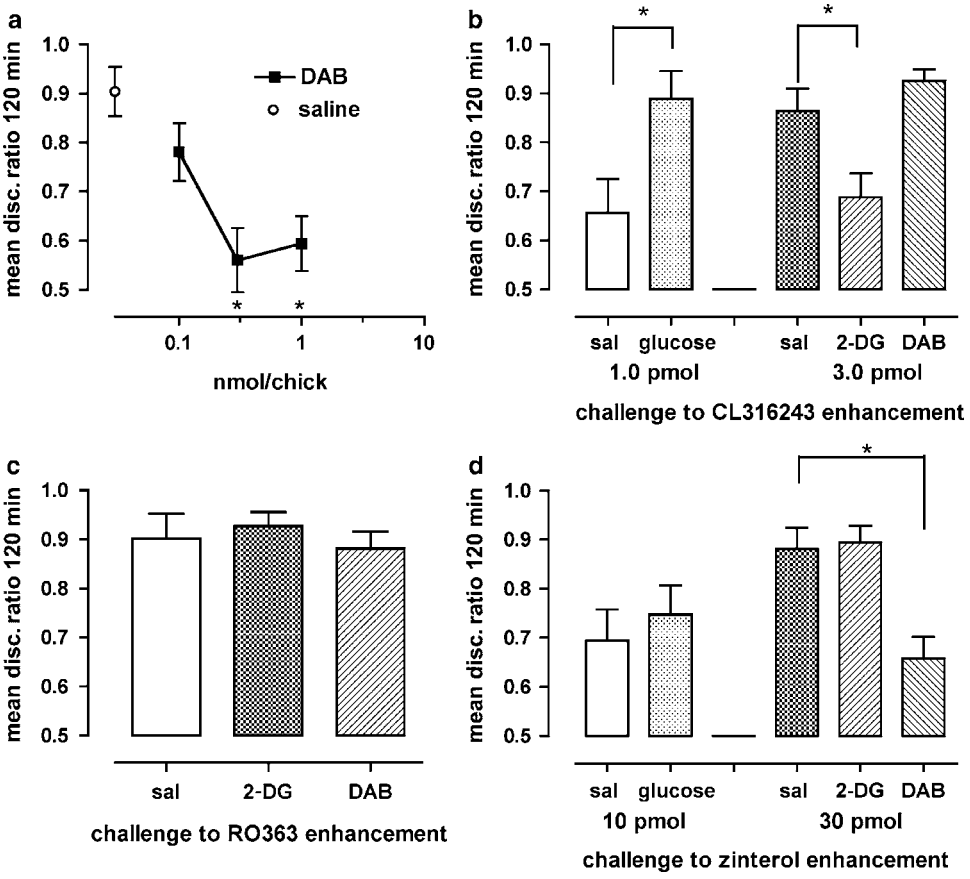

Figure 12 Role of hippocampal metabolism in β_2- and β_3-AR enhancement of weakly reinforced memory. (a) Dose–responses to subcutaneous doses of DAB to determine the low doses to be used in challenges. (b) Challenge of hippocampal β_3-AR agonist CL316243 (1.0 pmol/hemisphere) by saline and subcutaneous glucose (0.3 mmol/chick) and a higher dose of CL316243 (3.0 pmol/hemisphere) by the inhibitors 2-DG (1.0 mmol/chick) and DAB (0.1 nmol/chick) administered subcutaneously 5 min before the hippocampal injection of CL316243. (c) Challenges of hippocampal β_1-AR agonist RO363 by saline, 2-DG, and DAB. (d) Challenges of hippocampal zinterol (10 pmol/hemisphere) by saline and glucose, and to higher doses of zinterol (30 pmol/hemisphere) by saline, 2-DG, and DAB. Glucose facilitated and 2-DG reduced the memory consolidation by CL316243, whereas only DAB had any effect on zinterol enhancement of memory consolidation. Neither DAB nor 2-DG influenced memory enhancement by RO363. *$p < 0.05$, $N = 13$–16 per data point.

antagonist was the same as that observed with injection into the medial striatum (Gibbs and Summers, 2005), although the time of test yields a very different picture. Injections of CGP20712A into the striatum cause memory loss within the time frame of STM (within 10 min of training), but injection into the hippocampus only causes memory loss 60 min after training. Injection of the β_1-AR antagonist into the IMM had no effect on memory processing at any time of injection up to 30 min after training (Gibbs and Summers, 2002a).

β_2-AR involvement in hippocampus and IMM. There were at least two major differences between the effects of β_2-ARs in memory processing in IMM and in the hippocampus. In IMM, zinterol enhanced memory when injected between 0 and 30 min after weakly reinforced training (Gibbs and Summers, 2000), whereas in the hippocampus consolidation was promoted by injection between 15 and 30 min after training. This suggested that the activation of β_2-ARs in the hippocampus was associated only with the first part of ITM and not STM. The other difference is in the time window when memory was inhibited by the selective β_2-AR antagonist ICI118551. In IMM, memory is vulnerable to interference by ICI118551 between 5 and 25 min after training (Gibbs and Summers, 2005) and memory loss occurs after 30 min (ME Gibbs, unpublished data) as it does with propranolol acting at β_2-ARs (Gibbs and Summers, 2005). In contrast, ICI118551 injected into the hippocampus inhibited memory only 25 min after training, and as mentioned above memory loss occurs after 60 min.

The similarities cannot be explained by leakage of the injected drugs from the injection site to IMM. A bilateral injection of 10 pmol/hemisphere of ICI118551 (1 μl) injected 3 mm forward of the hippocampal injection site, 1–1.5 mm left and right of the midline, and at the same depth (1 mm) did not affect memory (DR: 0.91 ± 0.062, $n = 13$).

β_3-AR involvement in hippocampus and IMM. The profile of memory modulation mediated by the β_3-AR in the hippocampus differed from that displayed by either the β_1- or the β_2-AR. However, in terms of the time windows for promotion of consolidation of weakly reinforced training and for inhibition of strongly reinforced training, there was a remarkable similarity to the pattern seen following injection of β_3-AR agonists into the IMM (Gibbs and Summers, 2000, 2005), except that memory loss occurred after 10 min. Nonetheless, memory loss following hippocampal injection of SR59230A occurred earlier than for any of the other β-AR antagonists. In IMM, activation of the β_3-AR is associated with glucose uptake, in both behavioral experiments (Gibbs and Summers, 2002b) and *in vitro* studies in astrocytic cell cultures (Gibbs *et al*, 2007; Hutchinson *et al*, 2007). Both β_2- and β_3-ARs are found on astrocytes in the chick forebrain (Hutchinson *et al*, 2007). The present results suggest that β_3-ARs are also associated with glucose transport and astrocytes in the hippocampus.

In terms of the time sequence, the relationship between IMM and the hippocampus is not clear at present, but it is most likely that many brain regions are activated at the time of information input related to the learning task, including the IMM and the hippocampus, and that the differences in vulnerability of the different regions relate to differences in the timing of noradrenergic modulation.

DISCUSSION

Noradrenaline has a role in memory processing in the avian brain, acting via all three β-ARs. Agonists and antagonists selective for each of the three β-AR subtypes influence memory processing in the hippocampus and reveal different temporal characteristics as to when memory is vulnerable and when the memory is enhanced or lost. β_1-ARs are clearly involved in hippocampal memory processing and stimulation or blockade close to the time of training, and the timing is virtually identical to that seen with manipulation of the NMDA-Rs. Despite the inhibition of processing by β_1-AR or NMDA blockade within 2.5 min of training, memory loss does not occur until after 60 min. The crossover inhibition of both RO363 by D-APV and NMDA by CGP20172A (Figure 9) strongly supports the idea that their actions are related. As NMDA-Rs are responsible for LTP, our results suggest that memory processing in the hippocampus requires both NMDA-R and β_1-AR activation for LTP. Although the time of vulnerability in the medial striatum (MSt) is the same as in the hippocampus, the timing of the memory loss is very different (Gibbs and Summers, 2005), suggesting that the cellular mechanism is unlikely to be the same in these two areas. Noradrenaline release at this early time after training, in both the MSt and the hippocampus, is most likely to be related to attentional/arousal factors and will be activated by the locus coeruleus (Swanson-Park *et al*, 1999). Although there are a number of reports linking β-ARs with induction of LTP (Thomas *et al*, 1996; Winder *et al*, 1999), the β-AR subtype responsible is not identified. However, β_1-ARs are intimately associated with NMDA-R (Vanhoose and Winder, 2003), and activation of β_1-ARs may be a physiological signal regulating LTP induction.

Activation of β_2-ARs has no effect during STM, but can enhance memory consolidation when the agonist is injected during the lifetime of the first phase of ITM, that is, between 15 and 30 min after training. Memory is only vulnerable to inhibition by the antagonist 25 min after training and memory loss occurs after 60 min. There are a number of findings that suggest that the involvement of β_2-ARs in the hippocampus and IMM differs in some aspects: the extent of the vulnerable period to inhibition to the β_2-AR antagonist and the time of memory loss. These differences between IMM and the hippocampus are also seen with the protein synthesis inhibitor anisomycin.

β_3-ARs are also involved in memory processing in the hippocampus as in IMM. Memory enhancement occurs with activation during the lifetime of labile memory, up to 30 min after training, and memory loss occurs with activation between 2.5 and 5 min after training. The only difference is that memory loss starts slightly later in the hippocampus. Despite the differences in vulnerability to interference and time of memory loss, the cellular mechanisms by which β_2- and β_3-ARs consolidate memory in the avian hippocampus appear to be the same as in the avian cortex (Gibbs *et al*, 2007). The effects appear to be metabolically based, in that the ability of β_2-AR agonists

to enhance memory is limited if breakdown of glycogen is prevented with DAB, whereas the effect of β_3-AR agonists can be inhibited by 2-DG. The effective times of injection of the β_3-AR antagonist and 2-DG, up to 30 min post-training, suggest a relationship between β_3-AR activation and glucose uptake, albeit with a difference at 5 min before training because of the time required for 2-DG to build up to a sufficient concentration to inhibit the glycolytic pathway. Both β_2- and β_3-ARs are found on astrocytes in the chick forebrain (Hutchinson *et al*, 2007), and we have behavioral and *in vitro* evidence from cell cultures that suggests that the β_3-AR effect is directly related to glucose transport and uptake into astrocytes (Gibbs *et al*, 2007).

The timing of the effects of the β_3-AR agonist and antagonist on memory is remarkably similar to that of glucose and 2-DG. Likewise, the β_2-AR antagonist inhibited memory within a similar time frame to both DAB and anisomycin. In the hippocampus, the metabolic challenges to the effects of CL316243 or zinterol by glucose, 2-DG, and DAB suggest that the β_3- and β_2-AR are linked to glycolysis and glycogenolysis, respectively.

Although it has been reported that hippocampal injection of glucose leads to memory enhancement (McNay *et al*, 2000; Krebs and Parent, 2005; Dash *et al*, 2006), this is the first report suggesting that interference with glycolysis by 2-DG or prevention of glycogen breakdown in the hippocampus inhibits memory processing. As glycogen in the brain is stored primarily in astrocytes, this provides more support for a role for hippocampal astrocytes in memory processing.

Astrocytes have long been considered to have important structural functions in the central nervous system, but more recently a crucial metabolic role has been identified both in neuronal functioning and in memory (Hertz and Zielke, 2004; Gibbs *et al*, 2006a, b; Hertz *et al*, 2007). However, glia can also modulate or even mediate synaptic plasticity in a wide range of neuronal situations (Todd *et al*, 2006). Astrocytes are critical for glutamate reuptake from the extracellular space, where it is converted to glutamine and returned to the neurones that cannot synthesize glutamate or GABA *de novo* (Hertz, 2006). Astrocytes also respond to neurotransmitters released by synaptic terminals, including both glutamatergic and modulatory neurotransmitters (Perea and Araque, 2006). Activation of astrocytic metabotropic receptors by glutamate leads to calcium elevation in the astrocyte that then can cause the release of chemical transmitters, including glutamate, ATP, and D-serine (Haydon and Carmignoto, 2006).

Roles for Noradrenergic β-ARs in Memory Processing in the Avian Hippocampus

Since the timing of β_1-AR involvement in memory processing is close to the learning trial and hence to the beginning of STM, β_1-AR involvement is likely to relate to arousal mechanisms that are necessary for learning (Berridge and Waterhouse, 2003). Noradrenergic innervation to the chick hippocampus originates in the locus coeruleus as in mammals (Loy *et al*, 1980; Takatsuki *et al*, 1981; Reiner *et al*, 1994), where hippocampal CA1, CA3, dentate gyrus, and subiculum regions each receive a discrete noradrenergic input (Loy *et al*, 1980). There is evidence in mammals that, during the early period following training, noradrenaline release occurs in forebrain areas controlled by the locus coeruleus, including the hippocampus (Sara *et al*, 1994; Berridge and Abercrombie, 1999; Devoto *et al*, 2005; Yavich *et al*, 2005). We have evidence that locus coeruleus activation is important both at this early period of memory processing and at the time of consolidation after learning. Interfering with noradrenergic or glutamatergic transmission 2.5 or 25 min after training (but not in between these two times) results in memory loss, presumably because the locus coeruleus input into other brain regions is prevented (ME Gibbs and RJ Summers, unpublished data).

There are potentially at least two possible roles for β_2-ARs in the hippocampus. One involves the breakdown of glycogen in astrocytes and the other relates to LTP. The requirement for hippocampal β_2-AR activation during ITM is similar to the requirement for β-AR activation and maintenance of late-LTP in the dentate gyrus (Harley, 1998; Swanson-Park *et al*, 1999; Straube *et al*, 2003; Reymann and Frey, 2007). β-AR activation is involved in reinforcement of LTP in the rat dentate gyrus by novelty (Straube and Frey, 2003), where an interval of 15 or 30 min between the onset of reinforcement (ability to explore) and LTP induction led to the reinforcement of early- to late-LTP, an effect blocked by propranolol or the protein synthesis inhibitor anisomycin. The current finding that β_2-ARs are involved in avian hippocampal memory, together with the finding that blockade of β_2-ARs in chick forebrain slices inhibits LTP (Bradley *et al*, 1995), suggests that β_2-ARs are involved in the induction of late-LTP.

Roles for Different β-ARs in Synaptic Plasticity (LTP)

The chick model establishes roles for different ARs in the modulation of memory with temporal and functional specificity. β_1-ARs are involved at the time of acquisition, but the impact is not seen 60 min after training, suggesting that their activation is associated with the induction of a later phase of LTP. The similarity of effects on memory of drugs acting at NMDA-R and β_1-ARs supports a role for both receptors. β_2-AR involvement in hippocampal memory takes place at the time of consolidation from ITM to LTM. Time-course experiments suggest roles for β_1-AR in the induction of early-LTP (LTP-1) and β_2-ARs in protein synthesis and intermediate-LTP (LTP-2). Both β_2-AR and β_3-ARs probably have effects on astrocytic metabolism demonstrated by the rightward shift in the response of the β_2-AR agonist to inhibition of glycogenolysis and by the rightward shift in the response of the β_3-AR agonist to 2-DG and the leftward shift to glucose.

Early-LTP and the involvement of β_1-ARs. β-AR activation appears to influence both early-LTP (LTP-1) and protein synthesis-dependent LTP-2 in mammalian CA1 (Gelinas and Nguyen, 2005). There are a number of reports of β_1-AR involvement in CA1. β_1-AR modulation of NMDA-R by glutamate induces LTP at a hippocampal synapse that is stronger and more prolonged when β_1-ARs are activated by noradrenaline at the same time in mammalian hippocampal CA1 activation (Winder *et al*, 1999). There is evidence that β_2-ARs acting on the CA1 pyramidal cells (Hillman *et al*, 2005) and β_1-ARs acting on CA3 (Jurgens *et al*, 2005) are

both responsible for noradrenergic modulation of LTP in the hippocampus. β-AR modulation is important for early and late phases of LTP in CA3 (Huang and Kandel, 1996).

Protein synthesis-dependent, intermediate LTP-2 and the involvement of β_2-ARs. Protein synthesis is required during early-LTP for the induction of late-LTP (LTP-2) in the dentate gyrus (Otani and Abraham, 1989; Otani *et al*, 1989; Straube *et al*, 2003) and CA1 (Manahan-Vaughan *et al*, 2000; Vickers *et al*, 2005). This protein synthesis-dependent late-LTP is maintained by pre-existing mRNA, but subsequent LTP (LTP-3) production requires increased gene expression (Vickers *et al*, 2005). The question is open as to whether the protein synthesis is required only to refill stores of 'housekeeping' proteins necessary for memory (Reymann and Frey, 2007).

Clearly, the response of the hippocampus to β-AR activation will be complex and may involve pyramidal cells and granule cells in CA1, CA3, and the dentate gyrus, as well as astrocytes and interneurones—all of which may play different roles in memory acquisition and consolidation. This study has attempted to provide a clearer picture and identify which β-AR subtypes are involved in the avian hippocampus and when in the sequence of memory processing they act. Knowledge of β-AR subtype influences on memory acquisition and consolidation not only expands our understanding of the AR influence in the hippocampus but also points out the importance of subtype specificity and timing, when examining AR modulation of LTP in the mammalian brain.

Memory formation is a complex process that requires different brain systems acting in concert. Physiological events in one brain area must impact on other areas because of the connectivity between all the areas involved in memory. Likewise, interruption of memory processing in one brain area is likely to impact on processing in other areas. The main events must involve an initial activation of glutamatergic receptors leading to LTP, at least in the cortex and hippocampus. The activity in these areas is then influenced by modulatory neurotransmitters, in particular noradrenaline, the level of release of which is increased by the salience of the experience. This determines whether the memory is to be retained beyond the labile memory period. Activation of β_1-ARs is important for the induction of some forms of LTP, and in this study we have shown a relationship between β_1-ARs and NMDA-Rs, which suggests a direct influence on neuronal activity. In contrast, modulation of the memory trace by β_2- and β_3-ARs is likely to involve astrocytic mechanisms that are critical to the consolidation of memory. The close association of astrocytes with the neuronal synapse and their metabolic profile suggests that astrocytes are intimately involved in memory storage (Hertz *et al*, 1996, 2003; Gibbs *et al*, 2006a). Modulation of memory processing by noradrenaline clearly occurs via a number of different mechanisms and involves both neurones and astrocytes.

ACKNOWLEDGEMENTS

This work was funded in part by NHRMC Project Grant 143664 (Gibbs and Summers), Monash University Strategic Grant (Hutchinson), and Monash Research Grants (Gibbs). Dana S Hutchinson is a CJ Martin Fellow of the NHMRC Australia. We thank DG Anderson for the autoradiographs presented in this paper.

DISCLOSURE/CONFLICT OF INTEREST

The authors have no conflict of interest to report, not any involvement to disclose, financial or otherwise, over the past 3 years, that may bias the conduct, interpretation, or presentation of this work.

REFERENCES

Atoji Y, Wild JM (2006). Anatomy of the avian hippocampal formation. *Rev Neurosci* **17**: 3–15.

Berridge CW, Abercrombie ED (1999). Relationship between locus coeruleus discharge rates and rates of noradrenaline release within neocortex as assessed by *in vivo* microdialysis. *Neuroscience* **93**: 1263–1270.

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

Booze RM, Crisostomo EA, Davis JN (1989). Species differences in the localization and number of CNS β-adrenergic receptors: rat versus guinea pig. *J Pharmacol Exp Ther* **249**: 911–920.

Bradley PM, Burns BD, Webb AC (1995). Noradrenaline and potentiation in the chick brain slice. *NeuroReport* **6**: 1501–1504.

Bramham CR, Bacher-Svendsen K, Sarvey JM (1997). LTP in the lateral perforant path is beta-adrenergic receptor dependent. *NeuroReport* **8**: 719–724.

Clayton N, Krebs JR (1994). Memory for spatial and object specific cues in food-storing and non-storing birds. *J Comp Physiol A* **174**: 371–379.

Colombo M, Broadbent N (2000). Is the avian hippocampus a functional homologue of the mammalian hippocampus? *Neurosci Biobehav Rev* **24**: 465–484.

Crowe SF, Ng KT, Gibbs ME (1989). Effect of retraining trials on memory consolidation in weakly reinforced learning. *Pharmacol Biochem Behav* **33**: 889–894.

Csillag A, Szekely AD, Davies DC (1994). Termination pattern of medial hyperstriatum ventrale efferents in the archistriatum of the domestic chick. *J Comp Neurol* **348**: 394–402.

Dash PK, Orsi SA, Moore AN (2006). Spatial memory formation and memory-enhancing effect of glucose involves activation of the tuberous sclerosis complex—mammalian target of rapamycin pathway. *J Neurosci* **26**: 8048–8056.

Devoto P, Flore G, Saba P, Fa M, Gessa GL (2005). Stimulation of the locus coeruleus elicits noradrenaline and dopamine release in the medial prefrontal and parietal cortex. *J Neurochem* **92**: 368–374.

Ding L, Perkel DJ (2004). Long-term potentiation in an avian basal ganglia nucleus essential for vocal learning. *J Neurosci* **24**: 488–494.

Erichsen JT, Bingman VP, Krebs JR (1991). The distribution of neuropeptides in the dorsomedial telencephalon of the pigeon (*Columba livia*): a basis for regional subdivisions. *J Comp Neurol* **314**: 478–492.

Fernandez-Lopez A, Revilla V, Candelas MA, Gonzalez-Gil J, Diaz A, Pazos A (1997). A comparative study of α_2- and β-adrenoceptor distribution in pigeon and chick brain. *Eur J Neurosci* **9**: 871–883.

Field SF, Rickard NS, Toukhsati SR, Gibbs ME (2007). Maternal hen calls modulate memory formation in the day-old chick: the role of noradrenaline. *Neurobiol Learn Mem* **88**: 321–330.

Gelinas JN, Nguyen PV (2005). β-Adrenergic receptor activation facilitates induction of a protein synthesis-dependent late phase of long-term potentiation. *J Neurosci* **25**: 3294–3303.

Gibbs ME, Anderson DG, Hertz L (2006a). Inhibition of glycogenolysis in astrocytes interrupts memory consolidation in young chicks. *Glia* **54**: 214–222.

Gibbs ME, Hutchinson DS, Summers RJ (2007). Role of β-adrenoceptors in memory consolidation: β_3-adrenoceptors act on glucose uptake and β_2-adrenoceptors on glycogenolysis. *Neuropsychopharmacology* 1–14 doi:10.1038/sj.npp.1301629.

Gibbs ME, Ng KT (1977). Psychobiology of memory: towards a model of memory formation. *Biobehav Rev* **1**: 113–136.

Gibbs ME, Ng KT (1979). Behavioural stages in memory formation. *Neurosci Lett* **13**: 279–283.

Gibbs ME, O'Dowd BS, Hertz E, Hertz L (2006b). Astrocytic energy metabolism consolidates memory in young chicks. *Neuroscience* **141**: 9–13.

Gibbs ME, Summers RJ (2000). Separate roles for β_2- and β_3-adrenoceptors in memory consolidation. *Neuroscience* **95**: 913–922.

Gibbs ME, Summers RJ (2002a). Role of adrenoceptor subtypes in memory consolidation. *Prog Neurobiol* **67**: 345–391.

Gibbs ME, Summers RJ (2002b). Effects of glucose and 2-deoxyglucose on memory formation in the chick: interaction with β_3-adrenoceptor agonists. *Neuroscience* **114**: 69–79.

Gibbs ME, Summers RJ (2003a). α_2-Adrenoceptors in the basal ganglia have a role in memory consolidation and reinforcement. *Neuropharmacol* **45**: 355–367.

Gibbs ME, Summers RJ (2003b). Locus coeruleus involvement in memory formation. *Proc Aust Neurosci Soc* **14**: 8.

Gibbs ME, Summers RJ (2005). Contrasting roles for β_1, β_2 and β_3-adrenoceptors in memory formation in the chick. *Neuroscience* **131**: 31–42.

Harley CA (1998). Noradrenergic long-term potentiation in the dentate gyrus. *Adv Pharmacol* **42**: 952–956.

Haydon PG, Carmignoto G (2006). Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev* **86**: 1009–1031.

Hertz L (2006). Glutamate, a neurotransmitter—and so much more. A synopsis of Wierzba III. *Neurochem Int* **48**: 416–425.

Hertz L, Gibbs ME, O'Dowd BS, Sedman GL, Robinson SR, Sykova E *et al* (1996). Astrocyte–neuron interaction during one-trial aversive learning in the neonate chick. *Neurosci Biobehav Rev* **20**: 537–551.

Hertz L, O'Dowd BS, Ng KT, Gibbs ME (2003). Reciprocal changes in forebrain contents of glycogen and of glutamate/glutamine during early memory consolidation in the day-old chick. *Brain Res* **994**: 226–233.

Hertz L, Peng L, Dienel GA (2007). Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. *J Cereb Blood Flow Metab* **27**: 219–249.

Hertz L, Zielke HR (2004). Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci* **27**: 735–743.

Hillman KL, Doze VA, Porter JE (2005). Functional characterization of the β-adrenergic receptor subtypes expressed by CA1 pyramidal cells in the rat hippocampus. *J Pharmacol Exp Ther* **314**: 561–567.

Huang YY, Kandel ER (1996). Modulation of both the early and the late phase of mossy fiber LTP by the activation of beta-adrenergic receptors. *Neuron* **16**: 611–617.

Hutchinson DS, Summers RJ, Gibbs ME (2007). Activation of β_2- and β_3-adrenoceptors stimulate glucose uptake in chick astrocytes by distinct mechanisms: a mechanism for memory enhancement? *J Neurochem* **103**: 997–1008.

Izquierdo I, Bevilaqua LR, Rossato JI, Bonini JS, Medina JH, Cammarota M (2006). Different molecular cascades in different sites of the brain control memory consolidation. *Trends Neurosci* **29**: 496–505.

Johnston ANB, Rose SPR (1998). Isolation-stress-induced facilitation of passive avoidance memory in the day-old chick. *Behav Neurosci* **112**: 929–936.

Jurgens CWD, Rau KE, Knudson CA, King JD, Carr PA, Porter JE *et al* (2005). β_1 Adrenergic receptor-mediated enhancement of hippocampal CA3 network activity. *J Pharmacol Exp Ther* **314**: 552–560.

Krebs DL, Parent MB (2005). The enhancing effects of hippocampal infusions of glucose are not restricted to spatial working memory. *Neurobiol Learn Mem* **83**: 168–172.

Lee DW, Miyasato LE, Clayton NS (1998). Neurobiological bases of spatial learning in the natural environment: neurogenesis and growth in the avian and mammalian hippocampus. *NeuroReport* **9**: R15–R27.

Loy R, Koziell DA, Lindsey JD, Moore RY (1980). Noradrenergic innervation of the adult rat hippocampal formation. *J Comp Neurol* **189**: 699–710.

Manahan-Vaughan D, Kulla A, Frey JU (2000). Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. *J Neurosci* **20**: 8572–8576.

Margrie TW, Rostas JA, Sah P (2000). Inhibition of transmitter release and long-term depression in the avian hippocampus. *Neurosci Lett* **284**: 17–20.

Margrie TW, Rostas JAP, Sah P (1998). Long-term potentiation of synaptic transmission in the avian hippocampus. *J Neurosci* **18**: 1207–1216.

Mark RF, Watts ME (1971). Drug inhibition of memory formation in chicks. Long-term memory. *Proc R Soc Lond B* **178**: 439–454.

McNay EC, Fries TM, Gold PE (2000). Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. *Proc Natl Acad Sci USA* **97**: 2881–2885.

Nicholas AP, Hokfelt T, Pieribone VA (1996). The distribution and significance of CNS adrenoceptors examined with *in situ* hybridisation. *Trends Pharmacol Sci* **17**: 245–255.

Nikolakopoulou AM, Davies HA, Stewart MG (2006). Passive avoidance training decreases synapse density in the hippocampus of the domestic chick. *Eur J Neurosci* **23**: 1054–1062.

Otani S, Abraham WC (1989). Inhibition of protein synthesis in the dentate gyrus, but not the entorhinal cortex, blocks maintenance of long-term potentiation in rats. *Neurosci Lett* **106**: 175–180.

Otani S, Marshall CJ, Tate WP, Goddard GV, Abraham WC (1989). Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. *Neuroscience* **28**: 519–526.

Perea G, Araque A (2006). Synaptic information processing by astrocytes. *J Physiol Paris* **99**: 92–97.

Perkel DJ, Farries MA, Luo M, Ding L (2002). Electrophysiological analysis of a songbird basal ganglia circuit essential for vocal plasticity. *Brain Res Bull* **57**: 529–532.

Rainbow TC, Parsons B, Wolfe BB (1984). Quantitative autoradiography of β_1- and β_2-adrenergic receptors in rat brain. *Proc Natl Acad Sci USA* **81**: 1585–1589.

Raymond CR (2007). LTP forms 1, 2 and 3: different mechanisms for the 'long' in long-term potentiation. *Trends Neurosci* **30**: 167–175.

Raymond CR, Redman SJ (2006). Spatial segregation of neuronal calcium signals encodes different forms of LTP in rat hippocampus. *J Physiol* **570**: 97–111.

Reiner A (2005). A new avian brain nomenclature: why, how and what. *Brain Res Bull* **66**: 317–331.

Reiner A, Karle EJ, Anderson KD, Medina L (1994). Catecholaminergic perikarya and fibers in the avian nervous system. In: Smeets WJ, Reiner A (eds). *Phylogeny and Development of the*

Catecholamine Systems in the CNS of Vertebrates. Cambridge University Press: Cambridge. pp 135–181.

Reymann KG, Frey JU (2007). The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications. *Neuropharmacology* **52**: 24–40.

Rose SPR (2000). God's organism? The chick as a model system for memory studies. *Learn Mem* **7**: 1–17.

Sandi C, Rose SP, Patterson TA (1992). Unilateral hippocampal lesions prevent recall of a passive avoidance task in day-old chicks. *Neurosci Lett* **141**: 255–258.

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

Slesinger PA, Lowenstein PR, Singer HS, Walker LC, Casanova MF, Price DL *et al* (1988). Development of β_1 and β_2 adrenergic receptors in baboon brain: an autoradiographic study using [^{125}I]iodocyanopindolol. *J Comp Neurol* **273**: 318–329.

Straube T, Frey JU (2003). Involvement of beta-adrenergic receptors in protein synthesis-dependent late long-term potentiation (LTP) in the dentate gyrus of freely moving rats: the critical role of the LTP induction strength. *Neuroscience* **119**: 473–479.

Straube T, Korz V, Balschun D, Frey JU (2003). Requirement of beta-adrenergic receptor activation and protein synthesis for LTP-reinforcement by novelty in rat dentate gyrus. *J Physiol* **552**: 953–960.

Summers RJ, Papaioannou M, Harris S, Evans BA (1995). Expression of β_3-adrenoceptors in rat brain. *Br J Pharmacol* **116**: 2547–2548.

Swanson-Park JL, Coussens CM, Mason-Parker SE, Raymond CR, Hargreaves EL, Dragunow M *et al* (1999). A double dissociation within the hippocampus of dopamine D_1/D_5 receptor and β-adrenergic receptor contributions to the persistence of long-term potentiation. *Neuroscience* **92**: 485–497.

Szekely AD (1999). The avian hippocampal formation: subdivision and connectivity. *Behav Brain Res* **98**: 219–225.

Szekely AD, Boxer MI, Stewart MG, Csillag A (1994). Connectivity of the lobus parolfactorius of the domestic chicken (*Gallus domesticus*): an anterograde and retrograde pathway tracing study. *J Comp Neurol* **348**: 374–393.

Takatsuki K, Shiosaka S, Inagaki S, Sakanaka M, Takagi H, Senba E *et al* (1981). Topographic atlas of somatostatin-containing neurons system in the avian brain in relation to catecholamine-containing neurons system. I. Telencephalon and diencephalon. *J Comp Neurol* **202**: 103–113.

Thomas MJ, Moody TD, Makhinson M, O'Dell TJ (1996). Activity-dependent beta-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region. *Neuron* **17**: 475–482.

Todd KJ, Serrano A, Lacaille JC, Robitaille R (2006). Glial cells in synaptic plasticity. *J Physiol Paris* **99**: 75–83.

Tommasi L, Gagliardo A, Andrew RJ, Vallortigara G (2003). Separate processing mechanisms for encoding of geometric and landmark information in the avian hippocampus. *Eur J Neurosci* **17**: 1695–1702.

Unal B, Bradley PM, Sahin B, Canan S, Aslan H, Kaplan S (2002). Estimation of numerical density and mean synaptic height in chick hippocampus 24 and 48 hours after passive avoidance training. *Dev Brain Res* **136**: 135–144.

Vanhoose AM, Winder DG (2003). NMDA and beta1-adrenergic receptors differentially signal phosphorylation of glutamate receptor type 1 in area CA1 of hippocampus. *J Neurosci* **23**: 5827–5834.

Vickers CA, Dickson KS, Wyllie DJ (2005). Induction and maintenance of late-phase long-term potentiation in isolated dendrites of rat hippocampal CA1 pyramidal neurones. *J Physiol* **568**: 803–813.

Watts ME, Mark RF (1971). Drug inhibition of memory formation in chicks. Short-term memory. *Proc R Soc Lond B* **178**: 455–464.

Wieraszko A, Ball GF (1991). Long-term enhancement of synaptic responses in the songbird hippocampus. *Brain Res* **538**: 102–106.

Winder DG, Martin KC, Muzzio IA, Rohrer D, Chruscinski A, Kobilka B *et al* (1999). ERK plays a regulatory role in induction of LTP by theta frequency stimulation and its modulation by beta-adrenergic receptors. *Neuron* **24**: 715–726.

Yavich L, Jakala P, Tanila H (2005). Noradrenaline overflow in mouse dentate gyrus following locus coeruleus and natural stimulation: real-time monitoring by *in vivo* voltammetry. *J Neurochem* **95**: 641–650.