

## OPPOSING ROLES FOR GABA<sub>A</sub> AND GABA<sub>C</sub> RECEPTORS IN SHORT-TERM MEMORY FORMATION IN YOUNG CHICKS

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**Abstract**—The inhibitory neurotransmitter GABA has both inhibitory and enhancing effects on short-term memory for a bead discrimination task in the young chick. Low doses of GABA (1–3 pmol/hemisphere) injected into the multimodal association area of the chick forebrain, inhibit strongly reinforced memory, whereas higher doses (30–100 pmol/hemisphere) enhance weakly reinforced memory. The effect of both high and low doses of GABA is clearly on short-term memory in terms of both the time of injection and in the time that the memory loss occurs. We argue on the basis of relative sensitivities to GABA and to selective GABA receptor antagonists that low doses of GABA act at GABA<sub>C</sub> receptors (EC<sub>50</sub> approximately 1 μM) and the higher doses of GABA act via GABA<sub>A</sub> receptors (EC<sub>50</sub> approximately 10 μM). The selective GABA<sub>A</sub> receptor antagonist bicuculline inhibited strongly reinforced memory in a dose and time dependent manner, whereas the selective GABA<sub>C</sub> receptor antagonists TPMPA and P4MPA enhanced weakly reinforced in a dose and time dependent manner. Confirmation that different levels of GABA affect different receptor subtypes was demonstrated by the shift in the GABA dose-response curves to the selective antagonists. It is clear that GABA is involved in the control of short-term memory formation and its action, enhancing or inhibiting, depends on the level of GABA released at the time of learning. © 2005 Published by Elsevier Ltd on behalf of IBRO.

**Key words:** GABA, GABA<sub>C</sub> receptor, GABA<sub>A</sub> receptor, short-term memory, chick, discriminated avoidance learning.

The neurotransmitter GABA is found primarily in inhibitory interneurons impinging on and modulating activity in the vertebrate brain. GABA is considered to play a controlling role on the balance of excitability and inhibitory states in the cortex and hippocampus and the interneurons involved are viewed as having an active role in information processing (Paulsen and Moser, 1998). GABA receptor agonists and antagonists have been variously reported to enhance or inhibit memory processing, but recently GABA has been implicated directly in cognitive processing (Leventhal et al.,

2003) where a relationship between declining GABA levels and old age was demonstrated. An increase in visual function and discriminatory ability was found when GABA receptors were activated in the visual cortex in monkeys (Leventhal et al., 2003).

There are three major classes of GABA receptors in the CNS: GABA<sub>A</sub>, GABA<sub>B</sub> and GABA<sub>C</sub>. The pharmacology of GABA<sub>A</sub> and GABA<sub>B</sub> receptors has been extensively investigated (Johnston, 1996a), but GABA<sub>C</sub> receptors have been less well studied (Johnston, 1996b; Chebib and Johnston, 2000). GABA<sub>A</sub> and GABA<sub>C</sub> are both ionotropic receptors and activate chloride channels. GABA<sub>A</sub> receptors are inhibited by the alkaloid bicuculline whereas GABA<sub>C</sub> receptors are not (Johnston, 1996b). GABA<sub>B</sub> receptors are metabotropic, transmembrane receptors coupled to second messengers. They are activated by baclofen and blocked by phaclofen but not blocked by bicuculline. Relatively high levels of the GABA<sub>C</sub> receptor subunits ρ1 and ρ2 have been described in chick brain using *in situ* hybridization and RT-PCR (Albrecht et al., 1997). With the exception of δ-subunit containing GABA<sub>A</sub> receptors (Brown et al., 2002), GABA is an order of magnitude more potent on GABA<sub>C</sub> receptors than on GABA<sub>A</sub> receptors (Chebib and Johnston, 2000). The GABA<sub>C</sub> receptor antagonist TPMPA ((1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid; Ragozzino et al., 1996) is some eight times more potent as an antagonist of human recombinant ρ1 than of ρ2 GABA<sub>C</sub> receptors (Chebib et al., 1998), while P4MPA ((piperidine-4-yl)methylphosphinic acid) shows similar potency as an antagonist of both ρ1 and ρ2 GABA<sub>C</sub> receptors (Johnston et al., 1998).

GABA is involved in many neurological and psychiatric disorders including the epilepsies and GABA uptake systems have recently become a therapeutic target for these disorders (Sarup et al., 2003). There have been a number of reports suggesting that removal of the influence of inhibitory GABA receptors (e.g. by bicuculline) leads to memory enhancement and conversely its activation by agents such as muscimol leads to memory inhibition. (Brioni and McGaugh, 1989; Brioni et al., 1989; Castellano et al., 1989; Izquierdo and Medina, 1991; Clements and Bourne, 1996) As all three GABA receptors are activated by the neurotransmitter, the question arises as to how is there selectivity in the action of GABA? We have recently demonstrated a role for GABA<sub>C</sub> receptors in cognitive processing using selective GABA<sub>C</sub> receptor antagonists (Johnston et al., 1998) which contrasts with the role of the selective GABA<sub>A</sub> receptor antagonist bicuculline on memory in the chick.

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**Abbreviations:** DR, discrimination ratio; EC<sub>50</sub>, concentration producing 50% maximal response; GABA, γ-aminobutyric acid; IMHV, intermediate medial hyperstriatum ventrale; IMM, intermediate medial mesopallium; ITM, intermediate memory; LTM, long-term memory; P4MPA, (piperidin-4-yl)methylphosphinic acid; STM, short-term memory; TPMPA, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid.

We have a model of memory formation in the chick based on behavioral and neuropharmacological experiments that outlines three sequential stages in memory formation derived from single trial passive and discriminated avoidance tasks (Gibbs and Ng, 1977; Gibbs and Summers, 2002). The task involves day-old chicks learning not to peck at a red bead but to continue pecking at a blue bead on test when the red bead was made to taste aversive on training (100% anthranilate). Establishment of memory follows a very reproducible time course: short-term memory (STM) lasting 10 min, is followed after a transient dip at 15 min, by intermediate memory (ITM) lasting from 20 to 50 min, and after a second transient dip at 55 min, by protein synthesis-dependent long-term memory (LTM). LTM formation is dependent upon consolidation of the memory trace at 30 min, a time point that corresponds to phase A at the beginning of ITM. The division of ITM into phase A and phase B has been established on pharmacological grounds (Gibbs and Ng, 1984). Consolidation does not occur if the aversive stimulus is weakened by reducing the anthranilate concentration from 100% to 20%. In this case memory is normal during the first 30 min after training but ITMB and subsequently LTM does not appear. In a series of recent papers (reviewed in Gibbs and Summers, 2002) we have established roles for five adrenoceptor subtypes in the modulation of memory by systematic investigation involving selective adrenoceptor agonists and antagonists and establishing the selectivity of the action of the agonists at the receptors. We have shown that adrenoceptors respond to the selective adrenoceptor agonists in different brain regions and that the receptors are inhibited by selective adrenoceptor antagonists at different times during the sequential stages of memory.

In this paper, we have used a similar approach to elucidate the action of the different GABA receptor subtypes. We examine the effect of central injection of GABA on memory and the action of selective GABA<sub>A</sub> and GABA<sub>C</sub> antagonists and their effects on exogenously administered GABA on memory in the chick. As with studies on the differential contributions GABA<sub>A</sub> and GABA<sub>C</sub> receptors to the actions of GABA in rat retinal slices by Euler and Wässle (1998), we used lower doses of GABA to activate GABA<sub>C</sub> receptors and higher doses to activate GABA<sub>A</sub> receptors, together with selective GABA<sub>A</sub> and GABA<sub>C</sub> receptor antagonists.

The procedures outlined in this paper are 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 minimise both the suffering and the number of animals used.

## EXPERIMENTAL PROCEDURES

### Animals

Up to 240 1- to 2-day old Black Australorp×White Leghorn male chickens were delivered from a local poultry farm (Research Poultry Pty. Ltd, Research, Victoria, Australia) on the morning of each experiment. Full details of the experimental protocol are to be found in Gibbs and Summers (2002). The chicks were placed in

pairs, given *ad libitum* access to chick crumbs scattered on the floor of their box. Between 16 and 20 chicks were allocated to each experimental group. At the end of each experiment animals may be excluded on the basis of not training or not pecking at the control bead on test.

### Drugs and injections

GABA, the selective GABA<sub>C</sub> antagonists TPMPA and P4MPA (prepared as described by Hanrahan et al., 2001) and the selective GABA<sub>A</sub> antagonist (–)-bicuculline (Sigma) were made up in sterile physiological saline. Intracranial injections were administered by freehand injection of 5 or 10 μl per hemisphere, aimed at the intermediate medial hyperstriatum ventrale (IMHV) or mesopallium (IMM) of the forebrain at a depth of 3.5–4 mm using a Hamilton Repeating Dispenser syringe, with a stop on the 27gauge needle to control the depth of injection. This area of the chick forebrain has been shown to be metabolically active following imprinting (McCabe and Horn, 1994) and after passive avoidance training (Rose and Csillag, 1985; Sedman et al., 1992). Dose-response curves were constructed to bicuculline and P4MPA for s.c. administration for specificity challenges. In these cases, the drugs were injected in 100 μl volumes into a fold of skin on the ventral surface of the chick. In this paper we will use the new nomenclature introduced by Reiner et al. (2004) to describe the brain regions in the bird.

### Learning paradigm

The procedures have been described in detail elsewhere (Gibbs and Ng, 1977; Gibbs and Summers, 2002). Briefly, chicks were exposed to novel objects coming into their cage, to start with small chrome 2 mm diameter beads, and then larger red- and blue-colored glass beads which had been dipped in water. This was to encourage the chicks to peck freely at beads presented to them and to avoid fear of strange objects coming into their cage. This procedure reduces the variability in subsequent tests and ensures that all chicks will peck at both colored beads. Training consisted of presentation of a red bead after dipping it in the chemical aversant, methyl anthranilate. After tasting anthranilate chicks typically shake their heads and wipe their beaks on the floor. Once they have registered the taste they will not peck at it again in the 10 s trial. On retention testing, the chicks are presented with a clean red bead, followed by a clean blue bead 2.5 min later, and allowed 10 s to peck at each bead. The number of pecks at the beads and the latency to the first peck, in all trials with the colored beads, are recorded by on-line computer. The drugs were administered at predetermined times relative to the learning trial and testing was also relative to the learning trial.

At the completion of the experiment, data were retrieved from the computer and results calculated. Chicks failing to peck at the red training bead or failing to peck at the blue bead on test were excluded from further analysis. Exposing chicks to the presentation of beads into their cage before the training trial reduces problems of generalized non-pecking of beads. The exclusion of chicks on the basis of failing to peck the training bead or the blue test bead is made at the conclusion of the experiment.

### Statistics

Memory was indexed by a discrimination ratio (DR) defined as the ratio of the number of pecks at the blue bead to the total number of pecks at the red and the blue bead, for any chick which pecked at the blue bead on the retention test (Ng and Gibbs, 1991). A DR approaching 1.0 indicates good memory and a tendency to avoid or reduce pecking at the red bead, whereas a DR approaching 0.5 indicates equal pecking at both red and blue beads. The number of pecks on the blue bead can reach up to 10 or more in the 10 s trial.

All statistical tests were carried out with type 1 error rate set at  $\alpha=0.05$ , and statistical values are reported to three decimal

places' accuracy. Where unequal sample sizes were deemed to be the result of factors not associated with the treatments, unweighted means analyses were used. The unequal sample sizes arise from failure of chicks to peck at the red bead during training or to peck at the blue bead on retention test. Data were analyzed by one and two-way ANOVAs using SPSS (Information Analysis Systems SPS Inc., Chicago, IL, USA).

### Experimental designs

To demonstrate enhancement of memory, chicks were trained with 20% anthranilate on the training red bead (weakly reinforced training). Chicks normally do not remember the aversive taste on the red bead 120 min later. Memory for the aversive nature of the bead with 20% anthranilate is normally remembered for 30 min after training. After this time the memory fades. This labile memory for the weakly reinforced bead can be enhanced and put into permanent storage by administration of various agents at the appropriate time after training provided the administration occurs before the labile memory fades. To determine when memory can be consolidated, the time of injection of the consolidating agent is varied and given at different times after training. The retention of the memory is then followed by testing different groups at stated times after training.

Where inhibition of memory is attempted by the use of a drug, the chicks are trained on 100% anthranilate (strongly reinforced training). The time of administration of the drug can be varied around the training trial to determine at what time the memory is susceptible to interference by that drug. Memory retention was followed by testing different groups of chicks at predetermined times after the training trial.

In the following experiments there were four experimental protocols: dose response relationships, time of injection to determine when memory was susceptible and time of retention with an effective dose to see at which stage of memory the effect occurred. Finally, to determine the specificity of drug action to GABA at the selected receptor, pre-administration of selective antagonists by s.c. administration at a low dose which did not alter memory on its own was used to challenge the dose response curve to GABA.

## RESULTS

### Effect of GABA on memory retention following training on 100% or 20% anthranilate

Low doses of GABA (1.0–3.0 pmol/hem) injected into the IMM 2.5 min after training inhibited memory for strongly reinforced training in a dose dependent manner (Fig. 1A;  $EC_{50}$  approximately 3.0 pmol/hem;  $F_{5,94}=7.143$ ;  $P<0.001$ ), whereas higher doses of GABA (10–100 pmol/hem) enhanced memory for weakly reinforced training (Fig. 1B,  $EC_{50}$  approximately 10–30 pmol/hem;  $F_{5,94}=5.232$ ;  $P<0.001$ ). As the  $EC_{50}$  for GABA<sub>C</sub> is approximately 1  $\mu$ M and GABA<sub>A</sub> approximately 10  $\mu$ M (Johnston et al., 2003) we attributed these two opposing effects of GABA to effects on the two ionotropic subtypes of GABA receptor. GABA<sub>C</sub> receptors are activated at lower doses of GABA, whereas higher doses of GABA activate GABA<sub>A</sub> receptors. These results suggested that activation of GABA<sub>C</sub> receptors inhibited memory, whereas activation of GABA<sub>A</sub> receptors enhanced memory formation.

The times of administration relative to the training trial at which memory formation was susceptible to modulation by GABA were the same, with the low dose of GABA (1 pmol/

hem) producing inhibition and the high dose of GABA (100 pmol/hem) producing memory enhancement with injection of GABA up to +2.5 min after training. Injected close to training, GABA inhibited memory formation with injections immediately (+0 min) and +2.5 min after strongly reinforced training (Fig. 1C;  $F_{8,142}=8.886$ ;  $P<0.001$ ) and enhancement with injection +0 and +2.5 min after weakly reinforced training (Fig. 1D;  $F_{9,162}=5.715$ ;  $P<0.001$ ). These times implied an effect on STM processing. Both the low and the high dose of GABA had an effect on memory retention following injection 25 min after training, suggesting that there was an additional effect of increased GABA levels on the consolidation of labile to permanent memory at around 30 min posttraining. This was not investigated further in the current study.

The interpretation of the effect of 1 pmol of GABA at +2.5 min being on STM was confirmed by testing chicks after training and we found memory loss is apparent from the earliest time of test at 10 min after training (Fig. 1E;  $F_{6,108}=2.614$ ;  $P=0.021$ ).

### Inhibition of memory by injection of the GABA<sub>A</sub> antagonist bicuculline

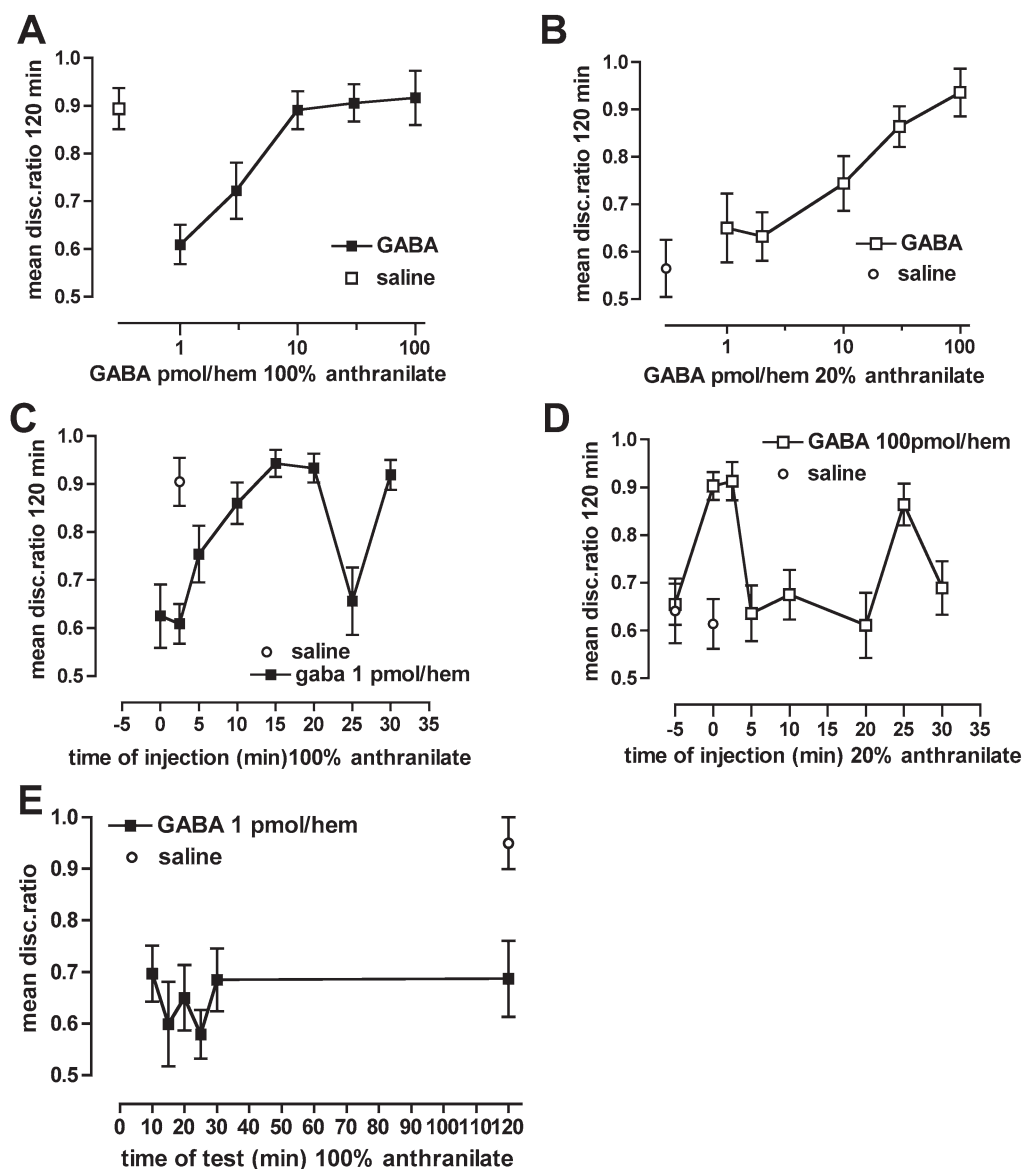
Injection of the GABA<sub>A</sub> receptor antagonist bicuculline produced a dose-dependent inhibition of memory expression following strongly reinforced training when injected into IMHV at either –5 min or +0 min relative to training (Fig. 2A), or injected s.c. at –5 min (Fig. 2B;  $F_{8,144}=4.533$ ;  $P<0.001$ ;  $F_{5,104}=12.008$ ;  $P<0.001$ ). Injection of bicuculline resulted in memory loss at 120 min when injected between –5 min and +2.5 min and again at +20 min ( $F_{9,156}=9.192$ ;  $P<0.001$ ; Fig. 2C). Bicuculline (10 pmol/hem) injected at +2.5 min resulted in memory loss by 5 min posttraining ( $F_{6,104}=2.270$ ;  $P=0.042$ ; Fig. 2D). The time of administration function (Fig. 2C) and the time of test function (Fig. 2D) closely resembled the effect of low doses of GABA (1 pmol/hem; Fig. 1C, E).

### Enhancement of memory by injection of the GABA<sub>C</sub> antagonists TPMPA and P4MPA

The selective GABA<sub>C</sub> antagonists TPMPA (Fig. 3A) and P4MPA (Fig. 3B) injected +2.5 min after weakly reinforced training enhanced memory in a dose dependent manner (Fig. 3A  $F_{6,106}=3.096$ ;  $P=0.008$ ; Fig. 3B  $F_{6,108}=32.64$ ;  $P<0.001$  respectively). The saline-injected controls showed the characteristic low levels of memory retention seen after weakly reinforced training. The times of injection around learning that promoted memory enhancement were the same as with the high dose of GABA (Fig. 1C, D) i.e. up to +2.5 min. TPMPA and saline injected at different times (Fig. 3C) showed a significant difference between saline and TPMPA injection at +0 and +2.5 min ( $F_{1,167}=8.10$ ;  $P=0.005$ ;  $F_{1,167}=10.36$ ;  $P=0.002$ ). P4MPA and saline (Fig. 3E) were also significantly different with injection at +0 and +2.5 min ( $F_{5,101}=4.054$ ;  $P=0.002$ ). For the time of test (Fig. 3E) there was a significant interaction between drug and test ( $F_{4,165}=4.06$ ;  $P=0.004$ ) with test times of 50, 60 and 120 min showing a significant difference between injection of saline and TPMPA. The s.c. dose response relationship for P4MPA showed 0.1 and 0.3 nmol/chick to

### Strongly-reinforced training GABA<sub>C</sub>-receptors

### Weakly-reinforced training GABA<sub>A</sub>-receptors



**Fig. 1.** Inhibition or enhancement of short-term memory by the neurotransmitter GABA on short-term memory for strongly (A, C, E) and weakly reinforced training (B, D). (A, B) Dose response relationships for GABA compared with saline injected 2.5 min after training. (C, D) Time of injection of GABA 1 or 100pmol/hem relative to training and tested 120 min later. (E) Memory retention in separate groups of chicks following 1 pmol GABA injected 2.5 min after training.

produce significant enhancement of memory compared with the lowest dose ( $F_{3,55}=13,851$ ;  $P<0.001$ ).

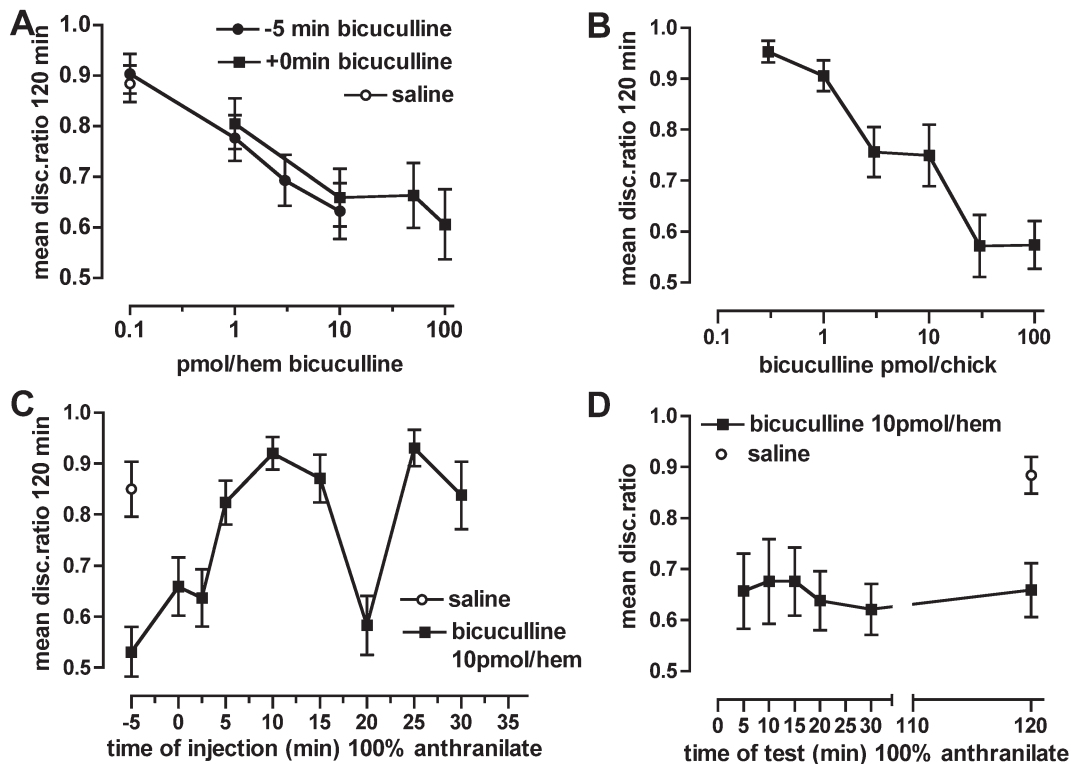
In support of the interpretation above, the GABA<sub>C</sub> receptor antagonists TPMPA and P4MPA injected into the IMM (Fig. 3A, B) or P4MPA injected s.c. (Fig. 3F) enhance the formation of memory injected +0 and +2.5 min after weakly reinforced training (Fig. 3C, D). Memory processing that was normally seen with strongly reinforced training was reinstated with injection of TPMPA 2.5 min after weakly reinforced training (Fig. 3E). STM lasting for 10 min

and ITM for 50 min were the same as normally seen with 100% anthranilate training.

#### Selectivity of action of GABA on GABA<sub>C</sub> and GABA<sub>A</sub> receptors

The specificity of action of low or high doses of GABA on GABA<sub>C</sub> and GABA<sub>A</sub> receptors was challenged by pre-administration of two doses of bicuculline. S.c. injection of bicuculline, at a dose that did not inhibit memory (1 pmol/

## Strongly-reinforced training GABA<sub>A</sub>-receptor antagonist inhibition of memory



**Fig. 2.** Inhibition of strongly reinforced memory by the selective GABA<sub>A</sub> receptor antagonist bicuculline. (A) Dose dependent inhibition following injection at 5 min before or immediately after training, or B after s.c. administration. (C) Time of injection (10 pmol/hem) of bicuculline with memory tested 120 min after training. (D) Memory tested in separate groups of chicks following bicuculline (10 pmol/hem) injected 2.5 min after training.

chick; Fig. 2B) was injected at  $-5$  min to challenge the inhibitory dose (1 pmol/hem; Fig. 4A) or the facilitatory dose (100 pmol/hem; Fig. 4B) of GABA administered at  $+2.5$  min. Bicuculline did not challenge the inhibitory effect of 1 pmol/hem of GABA ( $F_{2,52}=0.316$ ;  $P=0.730$ ), but the memory enhancing effect of 100 pmol/hem GABA was inhibited in a dose-dependent manner ( $F_{2,52}=10.296$ ;  $P<0.001$ ).

Further exploration of these findings showed that pre-administration of low doses of the GABA receptor antagonists P4MPA and bicuculline shifted the dose-response curves to GABA to the right. Therefore, GABA was less effective in the presence of a low dose of the selective GABA antagonists.

The dose-response curve for the lower doses of GABA with strongly reinforced training, was shifted to the right by prior administration of a low dose of P4MPA (Fig. 5A). There was a significant drug effect between pre-treatment with P4MPA and saline for the lower doses of GABA ( $F_{1,90}=14.25$ ,  $P<0.001$ ), and also for the higher doses of GABA between pre-treatment with bicuculline and saline ( $F_{1,106}=9.65$ ;  $P=0.002$ ).

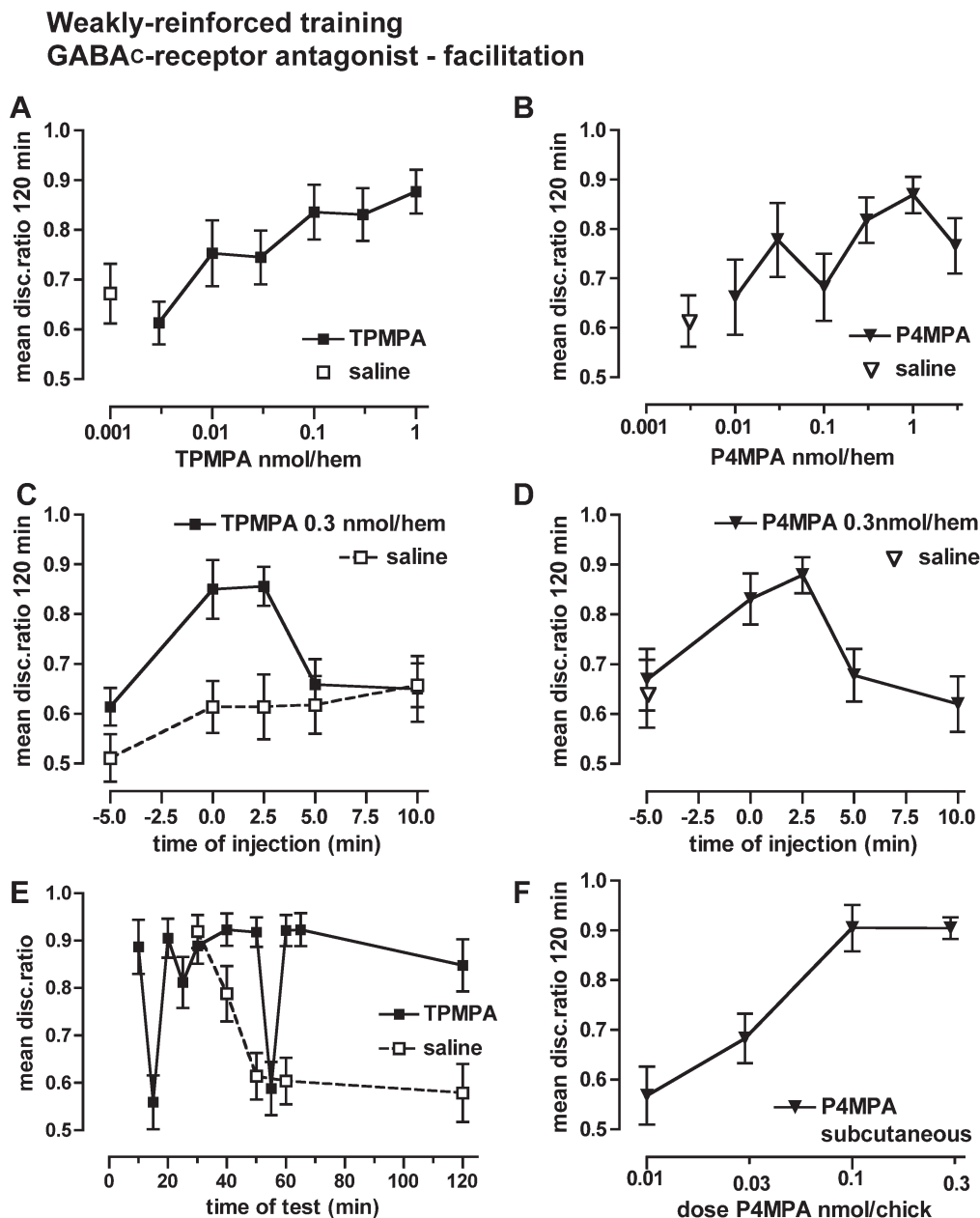
When the antagonists were switched around (Fig. 5B), the low dose of bicuculline did not shift the dose response to low doses of 0.3–1.0 pmol/hem GABA ( $F_{1,125}=1.17$ ;  $P=0.282$ ). Neither did pre-administration of a low dose

P4MPA shift the dose response to higher doses of GABA (3–100 pmol/hem;  $F_{1,119}=2.27$ ;  $P=0.135$ ). At 3 pmol/hem of GABA there was an apparent conflict in the action of P4MPA, perhaps due to GABA acting on both receptor subtypes (Fig. 5A).

The action of GABA on memory formation is obviously quite complex. It appears that endogenous release of GABA is necessary for memory formation and the action depends on the extracellular concentration, but what the 'normal' endogenous release of GABA is with weak or strong training is yet to be determined.

## DISCUSSION

From our results it is clear that in the chick forebrain multimodal association area (IMM or IMHV), GABA has two different effects on memory depending on the dose of the neurotransmitter injected. Low doses of GABA inhibit memory, whereas higher doses result in memory enhancement (Fig. 6). The effect is attributed to STM. The same pattern of memory loss is seen with injection of a relatively large dose of glutamate (40 nmol/hemisphere) and when astrocytic glutamate uptake is blocked by L-acetic acid  $\beta$ -hydroxamate (Gibbs et al., 2004). Blocking glutamate removal will result in an accumulation of extracellular glutamate from endogenous

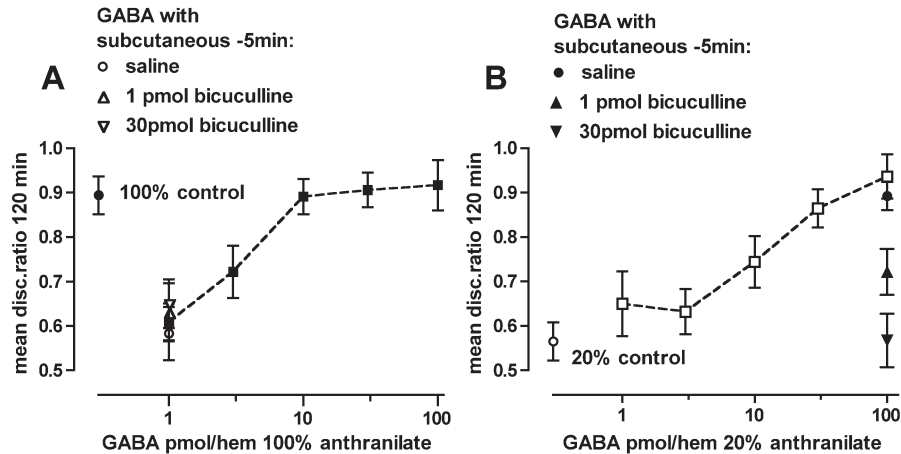


**Fig. 3.** Enhancement of memory by selective GABA<sub>C</sub> receptor antagonists following weakly reinforced training. (A, B) Dose response data for TPMPA and P4MPA injected 2.5 min after training. (C, D) Times of injection for TPMPA and P4MPA and saline relative to the training trial, memory tested 120 min after training; (E) memory retention tested in separate groups of chicks at different times after training following TPMPA 2.5 min after training and compared with chicks injected with saline at the same time. (F) Dose response relationship for s.c. administration of P4MPA injected 2.5 min and tested 120 min after training.

release and in both cases there is loss of STM with injections up to 2.5 min after training. There is a second time of susceptibility to GABA agents 25–30 min after training where a disturbance to GABA transmission also influences memory consolidation, but as yet we have not further explored this finding. It should be emphasized that for the GABA<sub>C</sub> receptor antagonist to have any effect on memory, there must be constitutive activity of GABA at the time of learning. Increased release of both GABA and glutamate in tissue slices of the chick IMHV

has been reported following passive avoidance training (Daisley and Rose, 2002).

The dose dependent effects of GABA on memory that we have observed in young chicks may result from the differential activation of GABA<sub>A</sub> and GABA<sub>C</sub> receptors as has been observed in the rat retina. Although GABA<sub>A</sub> and GABA<sub>C</sub> receptors both gate chloride channels, they are pharmacologically, molecularly and functionally distinct (Lukasiewicz, 1996; Chebib and Johnston, 2000). GABA<sub>C</sub> receptors were first cloned from the retina where they are



**Fig. 4.** Bicuculline challenge to inhibitory or facilitatory effect of GABA. (A) The inhibitory effect of 1 pmol/hem of GABA is not affected by prior s.c. injection of bicuculline. (B) The facilitatory action of 100 pmol/hemisphere of GABA is shifted in a dose-response manner by prior injection of bicuculline. S.c. doses of bicuculline from Fig. 2B.

found only on a subset of neurons whereas GABA<sub>A</sub> receptors are much more widespread. GABA<sub>A</sub> and GABA<sub>C</sub> receptors are considered to subservise different physiological functions in controlling visual transduction in the retina (Feigenspan and Bormann, 1994; Euler and Wässle, 1998). GABA<sub>A</sub> and GABA<sub>C</sub> receptors antagonists have opposite effects on the  $\beta$ -wave of the ERG recorded from isolated rat retina (Kapousta-Bruneau, 2000).

#### GABA<sub>A</sub> receptors

We have attributed the facilitating effects of high doses of GABA to an effect on GABA<sub>A</sub>-receptors. Several areas of the chick brain associated with learning have been shown to have a high density of GABA<sub>A</sub> receptors or are involved in its synthesis (see Harvey et al., 1998). GABA had to be injected within 2.5 min of training to have an effect. STM was inhibited by the selective GABA<sub>A</sub> receptor antagonist bicuculline injected within the same time frame, that is, no later than 2.5 min after training. When STM is inhibited by bicuculline, memory loss occurs within 5 min of the learning trial. The effect of the high dose of GABA is due to activation of GABA<sub>A</sub> receptors because the enhancing effect of 100 pmol/hem of GABA was reduced in a dose-dependent manner by prior administration of bicuculline in a dose-dependent manner. Specificity of action of high doses of GABA on GABA<sub>A</sub> receptors is demonstrated when prior administration of a low dose of bicuculline requires a higher dose of GABA to enhance memory. Prior administration of P4MPA did not change the dose response to high doses of GABA. We interpret this on the basis of a much higher density of GABA<sub>A</sub> receptors than of GABA<sub>C</sub> receptors such that at high doses of GABA any GABA<sub>C</sub> receptor response is overwhelmed by the response to GABA<sub>A</sub> receptors.

#### GABA<sub>C</sub> receptors

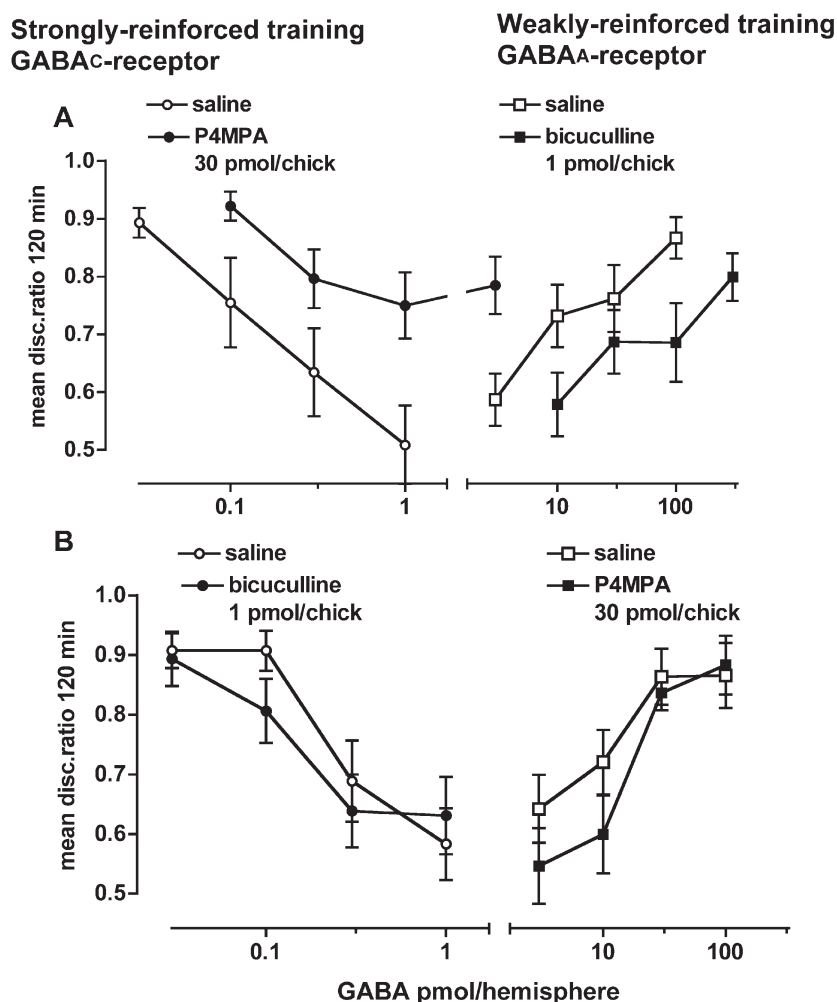
High levels of GABA<sub>C</sub> receptors (both  $\rho 1$  and  $\rho 2$  subtypes) are known to be expressed in chick brain (Albrecht et al., 1997). Although Albrecht et al. (1997) did not detect

GABA<sub>C</sub> receptors in the chick forebrain multimodal association area using their *in situ* hybridization methodology, this methodology was not sufficiently sensitive to detect GABA<sub>C</sub> receptors in rat brain, where they showed the presence of mRNA for GABA<sub>C</sub> receptors using RT-PCR. From our studies it seems likely that in the chick forebrain multimodal association area contains receptors that respond to low doses of GABA in a manner that can be antagonized by the GABA<sub>C</sub> receptor antagonists TPMPA and P4MPA and are insensitive to the GABA<sub>A</sub> receptor antagonist bicuculline suggesting the presence of functional GABA<sub>C</sub> receptors.

Low doses of GABA influence memory via activation of GABA<sub>C</sub> receptors. Injection of low doses (0.3–1.0 pmol/hemisphere) of GABA inhibited strongly reinforced memory. Like the higher doses, the low doses of GABA must be injected within 2.5 min of training to have an effect and memory loss is apparent soon after training. STM was enhanced by the selective GABA<sub>C</sub> receptor antagonists TPMPA and P4MPA as long as they were injected no later than 2.5 min after training. When STM was enhanced by a low dose of TPMPA, memory was reinstated within 10 min of the learning trial and the time course became the same as that of saline-treated chicks given strongly reinforced training.

The inhibitory effect of the low dose of GABA is related to the activation of bicuculline-insensitive GABA<sub>C</sub> receptors. This is supported by the finding that the effect of 1 pmol/hem of GABA is not affected by prior administration of bicuculline. Specificity of action of low doses of GABA is demonstrated with prior administration of a low dose of P4MPA requiring a higher dose of GABA to inhibit memory. Bicuculline did not change the dose response to low doses of GABA.

There is an effect of both high and low doses of GABA on STM in terms of both the time of injection necessary to produce the effect and the time over which the memory loss occurs. The EC<sub>50</sub> values for the ionotropic GABA<sub>C</sub> (approximately 1  $\mu$ M) and GABA<sub>A</sub> receptors (approx-



**Fig. 5.** Specificity of action of GABA at low doses on GABA<sub>C</sub>, and at higher doses, on GABA<sub>A</sub> receptors. (A) The dose response relationship of low doses of GABA on strongly reinforced training is shifted to the right in the presence of a low dose of the GABA<sub>C</sub> receptor antagonist P4MPA (30 pmol/chick). The dose-response relationship to the higher doses of GABA with weakly reinforced training is shifted to the right in the presence of the GABA<sub>A</sub> receptor antagonist bicuculline (1 pmol/chick). (B) The dose-response relationship to low doses of GABA constructed in the presence of bicuculline or the higher doses in the presence of P4MPA; there is no shift in the dose response curves.

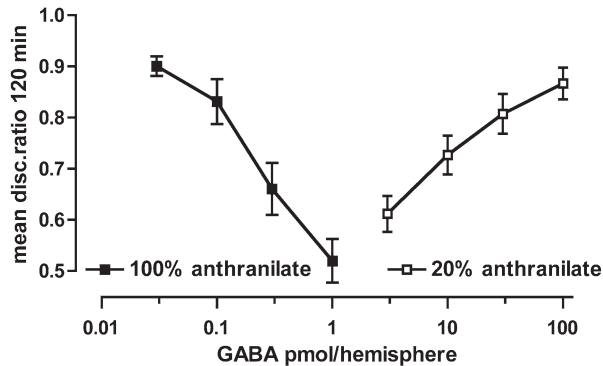
mately 10  $\mu$ M) supports our interpretation that the action of the low dose is via bicuculline-insensitive GABA<sub>C</sub>-receptors, whereas the higher dose acts via TPMPA-insensitive GABA<sub>A</sub>-receptors. It is clear that GABA is involved in the control of STM formation and that its effect depends on the level of GABA released at the time of learning.

### Role of GABA in memory

As mentioned in the introduction there have been a number of reports showing the opposite results to those reported here with respect to bicuculline, and suggesting that removal of the influence of inhibitory GABA receptors leads to memory enhancement and conversely the activation leads to memory inhibition. For example, inhibition by systemic or intra-amygdala bicuculline enhanced memory processing in aversively motivated tasks in chicks or rats and injection of the GABA<sub>A/C</sub> receptor agonist muscimol (like GABA, muscimol is

more potent at GABA<sub>C</sub> receptors than at GABA<sub>A</sub> receptors, Johnston et al., 2003) resulted in memory impairment (Brioni and McGaugh, 1989; Brioni et al., 1989; Castellano et al., 1989; Izquierdo and Medina, 1991; Clements and Bourne, 1996). Recent work with  $\alpha$ 5 subunit-containing GABA<sub>A</sub> receptor knockout mice showed better spatial memory than wild type mice (Collinson et al., 2002) and  $\alpha$ 5 inverse agonists are cognitive enhancers without being convulsants or increasing anxiety (Maubach, 2003). However, results similar to ours have also been reported where GABAergic antagonists injected into the striatum or substantia nigra produce amnesia (Chavez et al., 1995; Cobos-Zapiain et al., 1996). We showed some time ago that bicuculline inhibited memory in chicks (Gibbs, 1991).

The involvement of GABA in memory processing is likely to be complex, with its action in one region influencing processing in other brain regions (Cahill and McGaugh, 1998). Three issues, which may relate to this problem of



**Fig. 6.** Results of both dose response relationships in Fig. 5 (in the presence of saline) combined to illustrate the inhibitory effect followed by the excitatory effect of GABA in the dose range of 0.03–100 pmol/hemisphere.

different results being reported with bicuculline are (ii) the location of the GABA receptors in the brain, (ii) the timing of drug application and (iii) the relatively low doses used in the current study compared with doses used by others in the literature. We only report here on the effect of GABA and its antagonists when injected into the IMM of the chick forebrain. The effects of these drugs may be different if administered into different brain regions, although our preliminary experiments (M. E. Gibbs, unpublished observations) show that bicuculline injected into the avian caudate putamen or medial striatum (medial striatum or lobus parolfactorius) produces data that is similar to that seen with administration into the IMM. In addition, in the IMM, the action of bicuculline, like that of glutamate (Gibbs et al., 2003), is primarily in the left hemisphere. The route of injection of the drugs, particularly of central injections versus systemic administration is also crucial in the interpretation of the results generated. It is important to know the distribution of these GABA receptor subtypes in the brain and whether they are located on neurones or astrocytes. In addition to the site of action being important, the time of injection relative to learning and the dose levels achieved are clearly important factors in determining the outcome of treatment. In the study of Clements and Bourne (1996) bicuculline and muscimol were not injected close to the training trial, but were injected 30 min prior to training (into the IMM). This disturbance 30 min prior to training could have altered the responsiveness of the receptors. We also found that bicuculline given 30 min before training leads to memory enhancement (M.E. Gibbs and G.A.R. Johnston, unpublished observations). However, it is unlikely that the drugs would remain at a sufficiently high enough concentration to affect neuronal processing for any length of time. The interaction between GABA and other neurotransmitters may also determine the memory outcome.

Because we have a well-established memory model, we can determine the effect of drugs given at discrete times on the normal sequence of memory processing and this should enable us to tease out the effect of GABA on memory functioning. Even though GABA is an inhibitory neurotransmitter, it could of course facilitate transmission

by removing presynaptic inhibition in some areas of the brain.

The present data support the notion that various components of the memory trace are amenable to modulation by different neurotransmitters and we believe that our work has shown ways in which the discordant GABA literature results may be reconciled. GABA has different effects on STM depending on the dose and presumably this reflects differing synaptic and extrasynaptic concentrations as the result of the training experience. It will be important to establish the distribution of these two receptor subtypes in the brain to elucidate the precise role of GABA in STM, but it is clear that GABA<sub>C</sub> receptor antagonists can block the inhibitory action of a low dose of GABA in this memory paradigm, while a GABA<sub>A</sub> receptor antagonist can block the excitatory action of higher doses of GABA.

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