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BRAIN RESEARCH

Stimulation of $GABA_B$ receptors in the basal forebrain selectively impairs working memory of rats in the double Y-maze

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Abstract

The present experiments were conducted to evaluate the possible contribution of GABAergic inputs to the basal forebrain in the region of the nucleus basalis magnocellularis (nbm) to memory. In two experiments, rats implanted with bilateral intra-nbm guide cannulae were trained in the double Y-maze task to perform working- and reference-memory components. Animals were placed in one of two start arms of the first "Y" and the reference-memory component required travelling to its central stem for food. Access to the second "Y" then was given and the working-memory component for Expt. 1 required travelling to the goal arm diagonally opposite the start arm in the first "Y" of that trial. In Expt. 2, the working-memory component required travelling to the goal arm opposite to the goal arm entered in the second "Y" on the preceding trial, with 0- and 15-s delays between trials. In Expt. 1, pretrained rats ($n = 8$) received the $GABA_A$ agonist, muscimol (0.1 μg in 0.5 μl), the $GABA_B$ agonist, R(+)-baclofen (0.01, 0.05 and 0.1 μg), and its less active enantiomer, S(−)-baclofen (0.1 μg), in a counterbalanced order with retraining to criterion between injections. In Expt. 2, pretrained rats ($n = 9$) received saline (0.5 μl), R(+)-baclofen (0.1 μg), the $GABA_B$ antagonist, phaclofen (1 μg), and R(+)-baclofen + phaclofen. Results of Expt. 1 revealed that intra-nbm muscimol and, in a dose-dependent manner, R(+)-baclofen differentially affected working but not reference memory. In Expt. 2, the differential mnemonic impairment produced by R(+)-baclofen was replicated and co-injection with phaclofen reversed this effect. A 15-s delay between trials significantly impaired working but not reference memory. Results suggest that both $GABA_A$ and $GABA_B$ receptors may be involved in modulating the possible mnemonic functions of nbm cholinergic neurons.

Key words: Baclofen; Double Y-maze; γ-Aminobutyric acid; Memory; Muscimol; Nucleus basalis magnocellularis; Phaclofen; Reference memory; Working memory

1. Introduction

The nucleus basalis magnocellularis (nbm), lying within the basal forebrain, contains large cholinergic neurons distributed in the ventral pallidum, sublenticular substantia innominata, globus pallidus, internal capsule and nucleus ansa lenticularis [49]. Nbm cells receive amygdaloid, cortical and striatal afferents and send efferents to the dorsolateral frontal and parietal cortex and to the basolateral amygdala [9,25,48–50]. Both clinical and experimental data have implicated nbm neurons in memory.

Postmortem studies have demonstated that degeneration of cholinergic cells within the nucleus basalis of Meynert, affecting projections to the cortex [16] and amygdala [41], is an important feature of Alzheimer disease neuropathology along with the classical plaques and tangles. Behaviourally, Alzheimer disease is characterized initially by marked loss of memory for recent events and subsequently by widespread cognitive decline [40].

Studies of animals with excitotoxic lesions in the nbm, the rodent homologue to the nucleus basalis of Meynert, have demonstrated relatively specific recent memory impairments, like those seen in Alzheimer disease [3,6,27,43]. Although excitotoxins are not specific for cholinergic cells and the mnemonic impairments they produce do not correlate with the level of decrease in cortical choline acetyltransferase (ChAT) [19,21], recent studies have shown that excitotoxins that produce the greatest ChAT depletion in the amygdala

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result in the largest mnemonic impairments [7,33]. Thus, nbm cholinergic neurons projecting to the amygdala may be involved critically in processes of memory [29].

Cholinergic replacement therapies have met with limited success in the treatment of Alzheimer disease [2,14,30,37]. However, Sarter et al. [37] have suggested that anticholinesterases lead to a reduction in synthesis and release of acetlycholine and may uncouple presynaptic activity from signal transmission; similarly, direct stimulation of cholinergic receptors with muscarinic agonists may mask the effects of endogenously released acetlycholine, thereby decreasing the signal-to-noise ratio. Possibly, greater therapeutic success would result from treatments that enhance the signal in cholinergic neurons.

One approach involves manipulation of the influence of γ-aminobutyric acid (GABA) on cholinergic cells in the nbm. Anatomical studies have revealed that amygdalopetal cholinergic cells of the nbm are innervated by GABAergic neurons from the ventral striatum [50]. In addition, cholinergic cells receive input from GABAergic interneurons [24].

At least two classes of GABA receptors have been identified, $GABA_A$ and $GABA_B$ [8,20]. While both types cause inhibition, they are coupled to different ionic mechanisms. $GABA_A$ receptors activate the benzodiazepine/barbiturate receptor-linked chloride channel, producing postsynaptic inhibition [26]. $GABA_B$ receptors operate via GTP-binding protein mechanisms [1] and adenylate cyclase activity [44]; their stimulation produces increased outward K^+ conductance [22] or decreased Ca^{2+} conductance [8]. Both $GABA_A$ and $GABA_B$ receptors are distributed throughout the rat nbm [13].

Pharmacological studies have demonstrated that activation of ventral striatal GABAergic neurons or intra-nbm injections of GABA or the $GABA_A$ agonist, muscimol, result in decreased acetylcholine release in the cortex [10,47]. $GABA_B$ receptor modulation of nbm cholinergic neurons has not been reported. These data suggest that manipulation of GABAergic neurons innervating the nbm may modulate cholinergic neuronal activity.

Injection of GABA or muscimol into the nbm also has been shown to affect memory in a number of paradigms including the radial maze [31], passive avoidance [31,35], conditional visual discrimination [18,34] and a five-choice serial reaction time task [34]. Using a double Y-maze task, Beninger et al. [5] recently demonstrated that bilateral nbm injections of muscimol produced a dose-dependent and bicuculline ($GABA_A$ antagonist)-reversible working-memory but not reference-memory impairment.

While the mnemonic effects of $GABA_B$ receptor activation in the nbm have not been examined directly, systemic injections of the $GABA_B$ agonist, baclofen, produced mixed results. Thus, Georgiev et al. [23] demonstrated improved retention in an active avoidance task while other researchers reported impaired retention of inhibitory avoidance responses [11,12,42]. An impairment of inhibitory avoidance also was seen after intra-amydaloid baclofen [11].

The purpose of the present study was to assess the mnemonic effects of intra-nbm injections of the $GABA_B$ receptor agonist, R(+)-baclofen. Memory was tested in the double Y-maze [5,6,32] using a procedure with distinct working- and reference-memory components that placed equal demands on motivation, perception and motor abilities. Thus, if a treatment was shown to selectively impair performance on the working-memory component, it was concluded that memory was affected. Using this task in the first experiment, it was found that, like muscimol, R(+)-baclofen, but not the less active isomer S(−)-baclofen, produced a selective and dose-dependent impairment of working memory. The second experiment replicated the mnemonic effect of R(+)-baclofen and evaluated the pharmacological specificity of the effect by co-treating animals with the $GABA_B$ antagonist, phaclofen.

2. Materials and methods

This research was conducted with due regard for the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University policy and was approved by the Queen's University Animal Care Committee.

2.1. Subjects

Male Sprague–Dawley rats (15 and 16 for Expts. 1 and 2, respectively) were purchased from Charles River, Canada. Rats (200–250 g at the time of arrival) were group-housed before surgery and individually housed after surgery in hanging wire cages in a temperature-controlled (21 ± 1°C) colony room maintained on a 12-h light/dark cycle (lights on at 06:30). Water was available ad libitum in the home cage and food was rationed daily to maintain the rats at 80% of their free-feeding weights, adjusted for growth.

2.2. Surgery

After 8–10 days of handling and acclimatization to the animal quarters, rats were anaesthetized with Na pentobarbital (Somnotol, 65 mg/kg i.p.) and implanted bilaterally with chronic indwelling guide cannulae (0.64 mm diameter) aimed 1 mm dorsal to the nbm. The level-head stereotaxic co-ordinates were 1.3 mm posterior to bregma, 2.6 mm lateral to the midline and 6.8 mm ventral to the surface of the skull [36]. The guide cannulae were fixed to the skull using four stainless steel screws and dental acrylic cement. Stainless steel wire pins (0.32 mm diameter) were inserted into each cannula to keep them patent between injections.

2.3. Intra-nbm injections

Injection cannulae, made of stainless steel tubing (0.32 mm diameter) cut to extend 1 mm beyond the guide cannulae tips, were

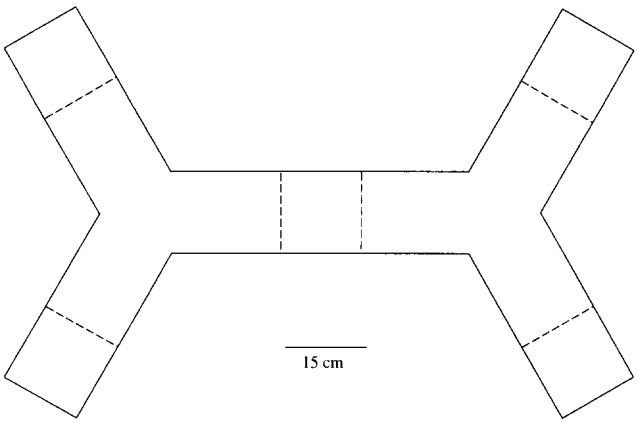


Fig. 1. Double Y-maze. Broken lines indicate manually operated barriers which could be used to restrict access to each part of maze. A trial began by placing rat in one of end arms and reference-memory choice was to go to goal box in stem. Access to second "Y" then was given and working-memory choice was to choose appropriate end arm. In Expt. 1, correct working-memory choice was to choose arm diagonally opposite starting position on that trial. In Expt. 2, correct working-memory choice was to choose alternate end arm from that chosen on previous trial.

attached by polyethylene tubing to two Hamilton microsyringes (10 μl), mounted on an infusion pump (Sage Instruments 355). Central infusions (0.5 μl/side) were made simultaneously at a constant rate of 0.5 μl/30 s. Injections were followed by a 60-s period during which injection cannulae were retained in place to ensure drug diffusion.

2.4. Drugs

All drugs were purchased from Research Biochemical (Natick, MA). Solutions of the $GABA_A$ agonist, muscimol hydrobromide (0.1 μg/0.5 μl), and the $GABA_B$ agonists, R(+)-baclofen hydrochloride (0.01, 0.05 and 0.1 μg/0.5 μl) and its less active enantiomer S(−)-baclofen hydrochloride (0.1 μg/0.5 μl), were prepared to the appropriate concentrations in physiological saline before behavioural testing. The $GABA_B$ antagonist, phaclofen (1 μg/0.5 μl), was dissolved in a mildly acidic 1 M HCl-saline solution (pH = 5) that then was neutralized with 1 M NaOH (pH = 7) and stored frozen for up to 8 wk. R(+)-baclofen was dissolved in the phaclofen solution to produce the R(+)-baclofen + phaclofen solution (0.1 μg + 1 μg/0.5 μl), then stored frozen for up to 8 wk.

2.5. Apparatus

The double Y-maze (Fig. 1) was composed of a central stem (55 cm long by 15 cm wide) with four arms (15 cm long by 15 cm wide), two at either end, each extending out at 120° angles. Removable wooden barriers (14 cm wide by 26 cm high), painted flat grey, could be inserted at the end of each arm and in the stem to provide five rectangular compartments (15 by 15 cm) and were used to permit or restrict entry into certain stems of the maze. The maze floor was constructed of parallel stainless steel bars, spaced ~1 cm apart, except that at the junction where the arms met the stem the floor was constructed of a triangular piece of Plexiglas. The maze walls (26 cm high) were wooden and painted flat grey. Plastic food containers were placed within the center of the rectangular areas in the four arms and the central stem. Quarter Froot Loop cereal pieces were placed in the food containers when used as reward and were scattered under the grid floor to mask possible odour cues. The maze was supported on a table (76 cm high) in a small room with a variety of visual cues (e.g. experimenter, lights and door frame) within sight of the maze.

2.6. Procedure

Pretraining: Food rationing began 5–7 days after surgery and animals reached their 80% target weights within 5 days. During this period, animals were handled and fed a small quantity (2 g) of Froot Loops daily in their home cage. Maze habituation then began during which the rats were placed in the maze for 20-min sessions, at roughly the same time daily, for 3 days. Animals were allowed free access to the maze with rewards placed in the centre and distal food containers.

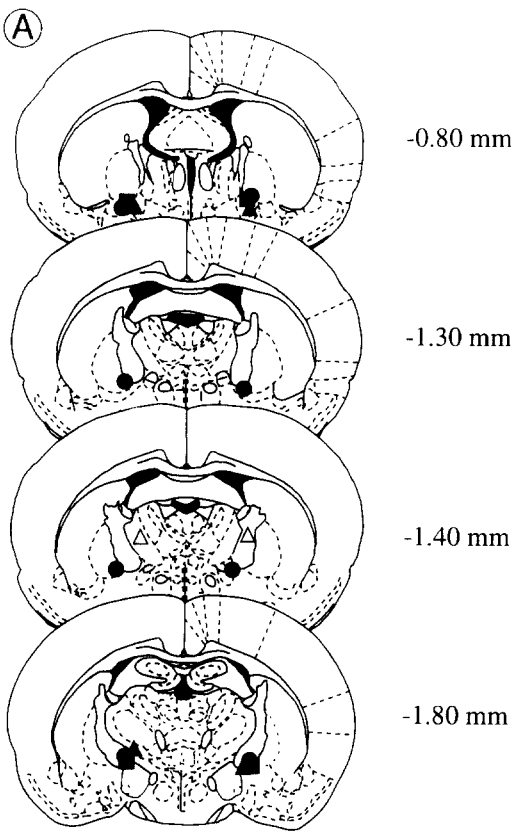
Training: At about the same time daily, 7 days per week, rats received ~40 trials until they began to acquire both components of the task (8–21 and 1–3 days in Expts. 1 and 2, respectively; see below). Animals then received 24 trials daily, unless performance dropped significantly, in which case an average of 40 trials (range 30–120) again was given daily until improvement was seen.

Each trial began by placing the rat, facing the back wall, in one of the two start arms of the first "Y". Rats were rewarded for exiting the arm and traveling to the goal box in the central stem, the distal end of which was blocked by a removable barrier. Upon consumption of the food reward in the central stem, a barrier was placed behind the rat, preventing its re-entry into the first "Y", and the previous barrier was removed, allowing the animal access to the second, identical, "Y". Rats again were rewarded for exiting the central stem, entering the correct final goal arm and traveling to its goal box. Once the food was consumed, the next trial began immediately. A choice was defined to have taken place when the animal's hind legs crossed onto the grid floor of an arm. Incorrect choices in the first or second "Y", or the correct choice and consumption of the food reward in the goal arm of the second "Y", resulted in the rat's immediate removal from the maze, after which another trial followed.

Expt. 1: The correct choice in the first "Y" required the use of reference memory. The animal always was to exit the start arm and enter the central stem of the first "Y", regardless of starting position. The start arm in the first "Y" varied randomly such that half of the trials began from each side, never more than three times in a row from that same side, within a given session. The correct choice in the second "Y" required the use of working memory. Rats were to exit the central stem of the second "Y" and enter the goal arm that was diagonally opposite to the start arm of the first "Y".

In the reference-memory component, entries into the arm of the first "Y" that had never been baited were scored as reference-memory errors. In the working-memory component, entries into the incorrect arm of the second "Y" (the arm directly rather than diagonally opposite the start arm in the first "Y" for that trial) were scored as working-memory errors. While, within a session, reference memory was scored out of 24 trials, the termination of a trial after a reference-memory error resulted in the number of working-memory trials being equal to 24 minus the number of reference-memory errors for each session. The numbers of reference- and working-memory errors were recorded daily and used to calculate the percentage of correct reference- and working-memory choices.

Training continued at 24 trials/day with a 0-s intertrial interval until choice accuracy reached a criterion of at least 85% correct (12–41 days) on both working- and reference-memory components averaged separately over a 3-day block. Animals were required to meet the additional condition that, on the 3rd day of criterion, performance be ≥88% correct for both working and reference memory to assure that they were performing at a high level before drug tests. Drug testing began once criterion was achieved.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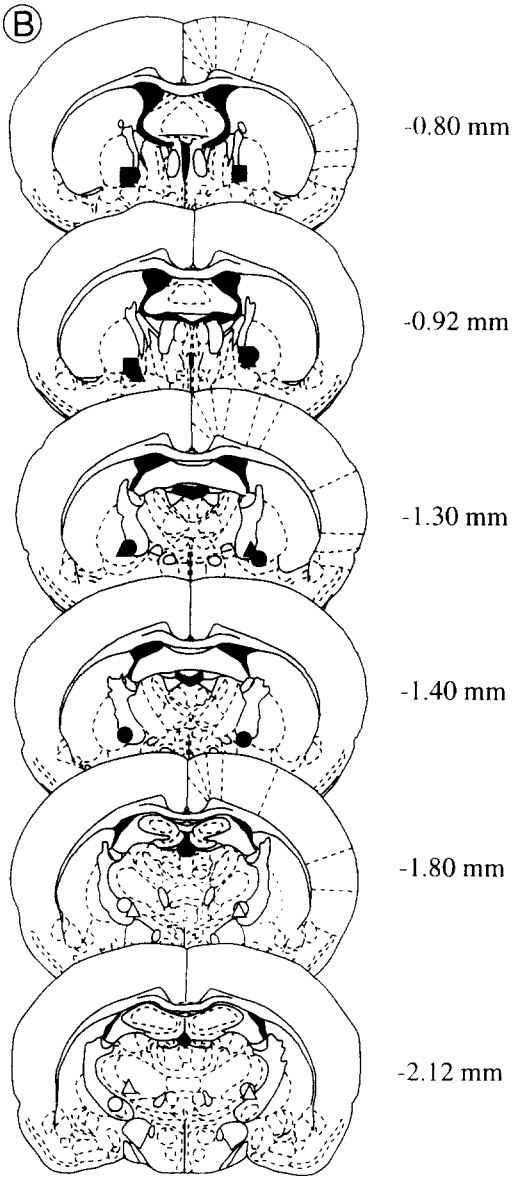
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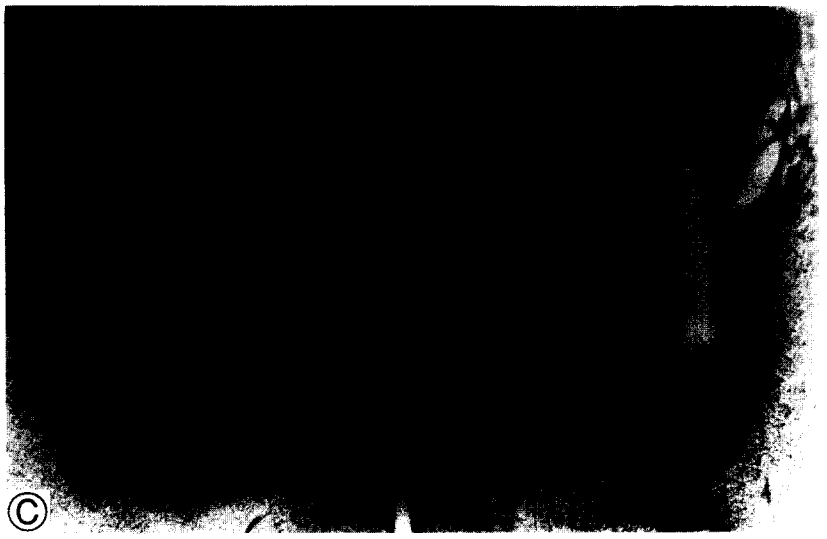
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A within-subjects design was used to examine the mnemonic effects of muscimol, R(+)-baclofen and S(−)-baclofen injections (0.5 μl) into the nbm. Each rat received five treatments, the order being counterbalanced across animals. The treatments were S(−)-baclofen (0.1 μg), muscimol (0.1 μg) and three doses of R(+)-baclofen (0.01, 0.05 and 0.1 μg). Between treatments, animals were retrained to criterion.

Expt. 2. The reference-memory component was identical with that used in Expt. 1. During training in Expt. 1, it was noted that rats tended to alternate their arm choice in the second "Y" from one trial to the next, before learning the correct working-memory response. In Expt. 2, this fact was used to produce a new working-memory rule in which correct performance required the rats to exit the central stem of the second "Y" and enter the goal arm opposite to the arm entered in the second "Y" on the previous trial, regardless of whether it had been correct or not. Thus, correct working-memory performance required the rats to alternate the final goal arm choice from trial to trial. On the rare occasion when a reference-memory error occurred, a free trial was given but neither the reference-memory nor the working-memory choice was scored. The correct working-memory choice for the following trial was based on the choice made on this free trial. This procedure was used to prevent an increase in errors on the working-memory component due to an increased delay between working-memory choices resulting from the time spent making the reference-memory error – a problem not present in Expt. 1 since each correct working-memory choice depended on the starting position for that particular trial.

All sessions began with a forced-trial in which one of the final arms of the second "Y" was randomly selected and blocked by a removable barrier, such that half of an animal's daily initial trials required entering one of the two final arms. This forced trial condition was implemented to prevent animals from choosing a start pattern and repeating it from session to session.

Scoring of reference-memory errors was similar to that used in Expt. 1. For the working-memory component, entries into the same final arm of the second "Y" entered on the preceding trial were scored as working-memory errors. As in the previous experiment, the numbers of reference- and working-memory errors were recorded daily and used to calculate the percentage of correct reference- and working-memory choices.

Training continued at 24 scored trials/day until choice accuracy reached a criterion of at least 90% correct (4–14 days) on both working- and reference-memory components averaged separately over a 3-day block. This assured a high level of performance before drug testing. Drug testing followed and sessions consisted of 12 randomly selected trials with a 0-s intertrial interval and 12 trials with a 15-s delay imposed between trials.

A within-subjects design was used to examine the mnemonic effects of delay and phaclofen, R(+)-baclofen + phaclofen and R(+)-baclofen injections (0.5 μl) into the nbm. Each rat received five treatments, the order of which was counterbalanced across animals. The treatments were no-injection, saline (0.5 μl), phaclofen (1 μg), R(+)-baclofen (0.1 μg) + phaclofen (1 μg) and R(+)-baclofen (0.1 μg). Animals received training between treatments until re-establishing criterion level performance.

2.7. Histology

After the completion of behavioural testing, the rats were injected with a lethal dose of Na pentobarbital and perfused intracardially with saline followed by 4% formalin solution. The brains were extracted and stored in a 4% formalin, 10% sucrose solution for at least 1 wk before being frozen and sliced into 50-μm coronal sections with a freezing microtome. The sections were mounted on glass slides and stained with thionin to verify cannula placements.

2.8. Statistical analyses

Percentage correct working- and reference-memory performance was analysed using a one-way (treatment) repeated measures ANOVA in Expt. 1 and a two-way (treatment × delay) repeated measures ANOVA in Expt. 2. The Greenhouse–Geisser adjusted df were employed to reduce possible type 1 error associated with repeated measures. In no case did the use of this more conservative procedure affect the outcome of the ANOVAs. To make the presentation of df in the statistical results more meaningful to the reader, unadjusted df are presented with F values but the P values given are those determined by the Greenhouse–Geisser adjustment. Dunnett's posthoc t tests, comparing each drug treatment to a control treatment, were conducted in both Expts. 1 and 2. In Expt. 1, the inactive [S(−)-] isomer of baclofen was used as the control treatment and, in Expt. 2, saline was used; each of these controls involved central administration of 0.5 μl of fluid but not a pharmacologically active compound. A Scheffé posthoc F test, evaluating all possible pairwise comparisons, was conducted to analyse the R(+)-baclofen dose response in Expt. 1. A planned t test, comparing R(+)-baclofen to R(+)-baclofen + phaclofen, was conducted in Expt. 2.

3. Results

3.1. Histology

Expt. 1: Of the original 15 animals, nine completed the experimental protocol. Of the six animals not completing the study, three rats discontinued eating and grooming after one or two central injections and subsequently died, and three rats dislodged their cannulae mounts before the completion of testing. Histological examinations carried out on the remaining nine animals revealed eight bilateral intra-nbm cannula injection sites, classified as hits, and one miss (Fig. 2A). Statistical analyses were conducted on the eight rats classified as hits.

Expt. 2: Of the original 16 animals, 13 completed the experimental protocol. Of the three animals not completing the study, two receiving R(+)-baclofen stopped eating and grooming and were sacrificed, and one animal failed to reach behavioural criterion. Histological examination was carried out on the remaining 13 animals and revealed nine hits and four misses (Fig. 2B). Statistical analyses were based on the data of the nine animals classified as hits.

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Fig. 2. Location of intracerebral injection sites. Position of cannula tips aimed at nbm for Expts. 1 and 2 are shown in A and B, respectively. Cannulae tracts for a representative animal are shown in C. Coronal sections were taken from Paxinos and Watson [36]. Anterior-posterior co-ordinates, relative to Bregma, are located to right of sections. Each rat was implanted with bilateral cannulae and shapes of symbols (circles, triangles and squares) indicate pairs of placements for a particular rat. Open symbols, misses; filled symbols hits.

3.2. Behaviour

In both experiments, animals were retrained after a central injection session until criterion was achieved again. This varied from rat to rat and from one criterion series of sessions to another and required a minimum of three sessions and a maximum of 21 sessions. There was no significant relationship between the treatment that preceded a series of sessions to criterion and the number of sessions required to reach criterion.

Expt. 1: The number of correct choices/session was converted to a percentage of total number of trials for each of the two memory components. Baseline scores were calculated by averaging the 3 criterion days preceding each of the five treatments into one working-memory and one reference-memory score for each animal. Thus, there were six scores/memory type for each rat: baseline, S(−)-baclofen (0.1 μg), muscimol (0.1 μg) and R(+)-baclofen doses of 0.01, 0.05 and 0.1 μg. Mean ± S.E.M. values are shown in Fig. 3.

Working memory was not impaired by S(−)-baclofen but was impaired by muscimol. Working memory appeared to have been impaired by R(+)-baclofen in a dose-dependent manner; 0.01 μg produced a small decrease, 0.05 μg produced a more pronounced decrease and 0.1 μg produced the largest decrease in the percentage of correct responses. Reference-memory choices were not impaired after any treatment. Thus, R(+)-baclofen and muscimol selectively impaired working-memory but not reference-memory scores.

Statistical analyses supported this description of the data. A one-way repeated measures ANOVA of the percentage correct working-memory choices revealed a significant treatment effect, $F_{5,35} = 19.82$, $P < 0.0001$. Dunnett's posthoc t tests, comparing each drug treatment to S(−)-baclofen, revealed that all drug treatments produced significantly lower percentage correct working-memory choices. Scheffé posthoc F tests, analysing all possible pairwise comparisons, revealed that the 0.1-μg R(+)-baclofen dose produced a significantly greater decrease in the percentage correct working-memory choices than the 0.01-μg dose. The percentage correct reference-memory responses was not affected by treatment as revealed by a one-way repeated measures ANOVA, $F_{5,35} = 0.95$, $P > 0.05$.

Expt. 2: The number of correct choices for each memory component and delay condition each day was converted to a percentage of the total number of trials of that type. There were 10 scores/memory type for each animal: no-injection, saline, phaclofen (1 μg), R(+)-baclofen (0.1 μg) + phaclofen (1 μg) and R(+)-baclofen (0.1 μg) for both the 0- and 15-s delays (Fig. 4).

Working-memory but not reference-memory scores were impaired by the 15-s delay condition (cf. Fig. 4B and D). As in Expt. 1, R(+)-baclofen (0.1 μg) decreased the percentage of correct working-memory choices. None of the other drug treatments appeared to affect working memory. Reference-memory scores were not affected by delay or drug treatment. Thus, a delay of 15 s or R(+)-baclofen impaired working but not reference memory. Furthermore, the mnemonic impairments produced by baclofen appeared to have been reversed by co-administration of the $GABA_B$ antagonist phaclofen.

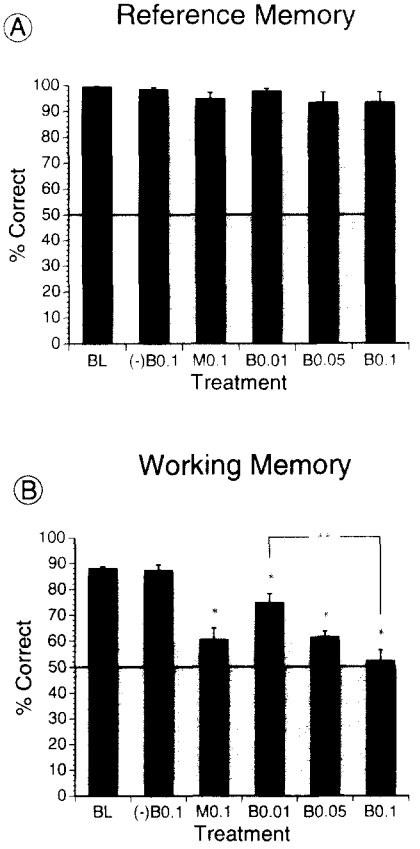

Fig. 3. Mean ± S.E.M. percentage correct reference- (A) and working-memory choices (B) in double Y-maze in Expt. 1. Data indicate baseline (BL) performance and effects of a 0.1-μg dose of S(−)-baclofen [(−)B0.1], muscimol (M0.1) and three doses of R(+)-baclofen (B0.01, B0.05 and B0.1). Horizontal line at 50% indicates chance performance. ANOVA revealed a significant ($P < 0.0001$) treatment effect for working memory only. * Significantly ($P < 0.05$) different from S(−)-baclofen control by Dunnett's tests. ** Significantly ($P < 0.01$) different from each other by a Scheffé test.

This description of the data was supported by statistical analyses. A two-way repeated measures ANOVA of the percentage correct working-memory choices revealed significant delay and treatment effects, $F_{1,8} = 36.46$, $P < 0.001$ and $F_{4,32} = 4.29$, $P < 0.05$, respectively. Dunnett's posthoc t tests, comparing each treatment to saline, revealed that R(+)-baclofen produced a significant decrease in the percentage of correct working-memory choices, $P < 0.01$. Planned comparison of the R(+)-baclofen alone treatment with the

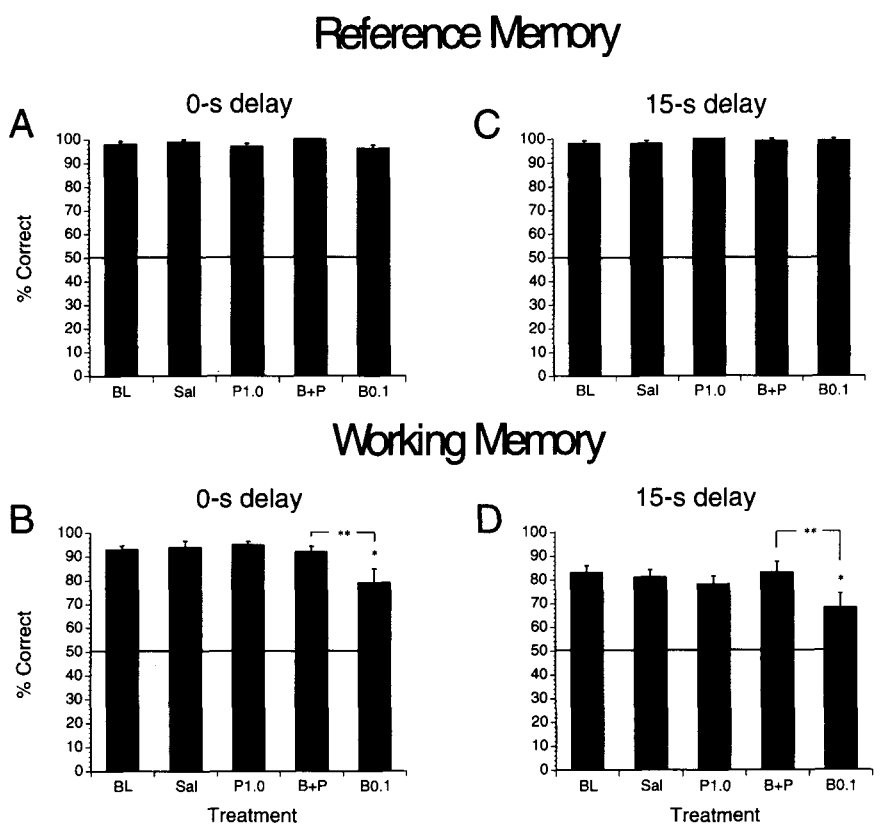

Fig. 4. Mean ± S.E.M. percentage correct reference- and working-memory choices in double Y-maze for 0-s delay condition (A and B, respectively) and 15-s delay condition (C and D, respectively) in Expt. 2. Data indicate baseline (BL) performance and effects of saline (Sal), phaclofen at a dose of 1 μg (P1.0), R(+)-baclofen + phaclofen (B + P) and R(+)-baclofen (B0.1). Horizontal line at 50% indicates chance performance. ANOVA revealed a significant treatment ($P < 0.05$) and delay ($P < 0.001$) effect for working memory only. * Significantly ($P < 0.05$) different from saline control by Dunnett's tests. ** Significantly ($P < 0.01$) different from each other by a planned t test.

R(+)-baclofen + phaclofen treatment revealed a significant difference, $t_8 = 7.04$, $P < 0.001$, showing that phaclofen reversed the impairment produced by R(+)-baclofen. Finally, there was no significant delay or treatment effect on the percentage correct reference-memory scores, $F_{1,8} = 3.26$, $P > 0.05$ and $F_{4,32} = 1.04$, $P > 0.05$, respectively.

4. Discussion

Results of Expt. 1 demonstrated that intra-nbm injections of the $GABA_A$ receptor agonist, muscimol (0.1 μg), selectively impaired working memory. This result replicated our previous findings [5]. The finding that bilateral intra-nbm administration of the $GABA_B$ agonist, R(+)-baclofen, but not its less active enantiomer, S(−)-baclofen, produced a dose-dependent and selective working-memory impairment has not been reported previously.

In Expt. 2, working-memory but not reference-memory impairments were shown to be delay-dependent. This result is in accordance with our previous findings [6,32] and verifies the differential nature of the mnemonic demands of the two components of the double Y-maze task. The impairment of working memory produced by R(+)-baclofen (0.1 μg) in Expt. 1 was replicated in Expt. 2. Further, results revealed that this impairment was reversed with co-administration of the $GABA_B$ antagonist, phaclofen (1 μg), demonstrating the $GABA_B$ receptor specificity of the R(+)-baclofen effect. When bilaterally administered into the nbm alone, the same dose of phaclofen had no significant effect on either memory type. Taken together, these data suggest that GABAergic activation of either $GABA_A$ or $GABA_B$ receptors in the nbm may influence mnemonic processes, possibly through direct or indirect modulation (see below) of cholinergic neurons.

There are several alternative explanations for the observed behavioural impairments in the present study [5]. If muscimol or R(+)-baclofen injected into the nbm induced a generalized motor hypoactivity, a resulting increase in travel time required to complete either memory component might selectively impair working memory since working memory has proven sensitive to delay manipulations [5,32]. Although latency data were not systematically collected in the present study, neither muscimol nor R(+)-baclofen

appeared to produce motor impairments at any dose that selectiviely affected working memory. This observation is supported indirectly by studies demonstrating *increased* locomotion in rats after ibotenic acid lesions of the nbm, or intra-nbm injections of muscimol (0.025–0.05 μg) [4,17,28,38,39]. Thus, bilateral nbm muscimol or R(+)-baclofen injections in the present study probably exerted their disruptive effects on memory rather than motoric processes.

If the level of component difficulty, as indicated by rate of acquisition, interacts with the mnemonic effects of GABAergic agents, differential difficulty in acquisition of the working- and reference-memory components may have contributed to the observed muscimol- or R(+)-baclofen-induced working-memory impairments. The reference-memory component was learned to criterion by all animals within the first few days whereas the working-memory component required many more sessions. It was also the case that the original working-memory rule in Expt. 1 required more sessions (12–41) before being learned to criterion than the modified working-memory rule in Expt. 2 (4–14 sessions). Results revealed that the working-memory impairment produced by R(+)-baclofen (0.1 μg) in Expt. 1 using the "difficult" rule was greater than the impairment in Expt. 2 using the "easy" rule (cf. Figs. 3 and 4A,B) even though both were significantly different from their respective controls. This might provide some support for the differential component difficulty hypothesis. It is not possible from the present results to rule out this hypothesis. Hepler et al. [27], however, have addressed this issue. Rats trained in a conditional discrimination task in which working- and reference-memory acquisitions were similar showed impairments of only working memory after excitotoxic lesions of the nbm. These results demonstrate that, while it can not be ruled out unequivocally, differential component difficulty is not a sufficient explanation for the selective working-memory impairments produced by manipulations of the nbm.

The results of the present study are in accord with other investigations of GABAergic mediation of mnemonic functions in the nbm. Thus, Majchrzak et al. [31] reported that performance in a radial maze task and acquisition of passive avoidance were impaired by chronic unilateral intra-nbm injections of GABA. Nagel and Huston [35] showed that posttrial bilateral infusions of muscimol into the nbm impaired performance in a passive avoidance task. In a conditional visual discrimination task, Dudchenko and Sarter [18] found that intra-nbm injections of muscimol dose-dependently impaired performance. Muir et al. [34] showed that muscimol injected bilaterally into the nbm disrupted performance of a five-choice serial reaction time task and a conditional visual discrimination task. Previously, we reported that intra-nbm injections of muscimol impaired working but not reference memory in a dose-dependent, and bicuculline-reversible, manner in a double Y-maze task [5]. Swartzwelder et al. [42] demonstrated that posttraining systemic injections of baclofen impaired 7-day retention of a one-trial passive avoidance task. Using a one-trial step-through inhibitory avoidance task, Castellano et al. [11] found that posttraining systemic or intra-amygdala injections of baclofen dose-dependently impaired 48-h retention. This effect was shown to be reversible with a low but otherwise ineffective dose of the cholinergic agonist oxotremorine [12]. The present observations add to these by showing a differential effect of muscimol (0.1 μg) and R(+)-baclofen (0.01, 0.05 and 0.1 μg) on working and reference memory, in a task that places equal motor, sensory and motivational demands on the two components, making nonmnemonic interpretations difficult. It can be concluded that GABAergic modulation of the nbm at both $GABA_A$ and $GABA_B$ receptors significantly influences memory.

While the location of $GABA_A$ receptors in the nbm is known [49], the location of $GABA_B$ receptors in the nbm which may be involved in memory is not clear. One possibility is that, like $GABA_A$ receptors [49], $GABA_B$ receptors may be located on cholinergic neurons, thereby directly inhibiting them. A second possibility is that $GABA_B$ receptors may indirectly inhibit cholinergic activity by inhibiting excitatory amygdalo- and/or corticofugal glutamatergic afferents to nbm cholinergic cells as is the case in the septal-hippocampal system [15,45,46]. Further studies addressing the location of possible memory-modulating $GABA_B$ receptors within the nbm are needed.

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5. References

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