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Basal forebrain injections of the benzodiazepine partial inverse agonist FG 7142 enhance memory of rats in the double Y-maze

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Abstract

Cholinergic replacement strategies have achieved little success in the treatment of Alzheimer's disease. It has been suggested that the mnemonic function of cholinergic neurons may be enhanced by treatments that reduce GABA-ergic inhibition, while preserving the normal pattern of activity in the cholinergic neurons. Following on these suggestions, the present study investigated the mnemonic effects of intra-nucleus basalis magnocellularis (NBM) injections of the benzodiazepine receptor partial inverse agonist *N*-methyl-β-carboline-3-carboxamide (FG 7142). Rats were surgically implanted with bilateral cannulae in the NBM prior to training in a double Y-maze. Daily training sessions continued until reference and working memory choice performance stabilized to a criterion of $\geq 91\%$ correct. Rats ($n = 9$) received FG 7142 bilaterally in doses of 0.2, 2.0 and 3.0 μg/0.5 μl per side, muscimol (a $GABA_A$ agonist) in a dose of 0.1 μg/0.5 μl per side, vehicle (345 μg 2-hydroxypropyl-β-cyclodextrin/0.5 μl saline per side) or no injection in a counterbalanced order with retraining to criterion between treatments. Muscimol impaired choice accuracy on both the reference and working memory components, but the effect was bigger for working memory, replicating our previous findings. Two doses of FG 7142 (0.2 and 2.0 μg/0.5 μl) enhanced choice accuracy on the working memory component. The present results suggest that benzodiazepine partial inverse agonists may enhance mnemonic function.

Keywords: FG 7142; Double Y-maze; γ-Aminobutyric acid; Memory; Muscimol; Nucleus basalis magnocellularis; Reference memory; Working memory

1. Introduction

The nucleus basalis magnocellularis (NBM), located in the basal forebrain, contains large cholinergic neurons distributed in the ventral pallidum, sublenticular substantia innominata, globus pallidus, internal capsule and nucleus ansa lenticularis [40]. NBM cells receive amygdaloid, cortical and striatal afferents and send efferents to the dorsolateral frontal and parietal cortex and to the basolateral amygdala [6,16,39,40]. Both experimental and clinical data have implicated NBM neurons in memory.

Post-mortem studies have shown that degeneration of cholinergic cells within the nucleus basalis of Meynert, affecting projections to the cortex [8] and amygdala [34], is an important feature of the neuropathology associated with Alzheimer's disease, along with the classical plaques and neurofibrillar tangles. Behaviourially, Alzheimer's disease is characterized initially by marked loss of memory for recent events and subsequently by widespread cognitive decline [33,37].

Studies of animals with excitotoxic lesions in the NBM, the structure in rodents homologous to the nucleus basalis of Meynert in humans, have demonstrated relatively specific recent or working memory impairments like those seen in the early stages of Alzheimer's disease [4,18,24,30]. Excitotoxins are not specific to cholinergic cells and recent studies have shown that the mnemonic impairments they produce do not correlate with the level of decrease in cortical choline acetyltransferase (ChAT) [12–13]. However, it has now been shown that the excitotoxins that produce the greatest ChAT depletion in the amygdala are those

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that result in the largest mnemonic impairments [5]. Thus, NBM cholinergic neurons projecting to the amygdala, as well as those projecting to the cortex, may be critically involved in processes of memory [3,19].

Cholinergic replacement therapies have met with limited success in the treatment of Alzheimer's disease [1,7,20,31]. However, Sarter et al. [31] have suggested that anticholinesterases lead to a reduction in synthesis and release of acetylcholine and may uncouple presynaptic activity from signal transmission; similarly, direct stimulation of cholinergic receptors with muscarinic agonists may mask the effects of endogenously released acetylcholine, thereby decreasing the signal-to-noise ratio. Possibly, greater therapeutic success would result from treatments that enhance the signal in cholinergic neurons. This might be achieved by reducing the level of inhibition of NBM cells.

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 [25,40]. In addition, cholinergic cells receive input from GABAergic interneurons [15]. Recently, Hannila et al. [17] have proposed that GABAergic neurons may be preserved in patients with Alzheimer's disease and that the remaining NBM cells may be subjected to increased GABAergic inhibition, further suggesting the possible therapeutic advantage of reducing GABAergic inhibition of NBM cells. The functional importance of GABAergic inhibition of NBM cholinergic cells has been shown in neuropharmacological and behavioral studies; thus, local injections of GABAergic agonists into the NBM reduced cortical release of acetylcholine [36] and impaired recent memory [2,9,11,22,27].

GABAergic neurotransmission also can be manipulated with compounds that influence the benzodiazepine (BZD) receptor. For example, systemic injection of the β-carboline ZK 93 426, a BZD receptor antagonist, lessened exploratory deficits in a radial maze induced by an excitotoxic lesion of the NBM [32]. Recently, Mayo and colleagues [23] reported that local injection of β-carboline-methyl beta carboline-3-carboxylate (β-CCM) – a full inverse agonist at BZD receptors – into the NBM of rats enhanced recognition performance in a two-trial recognition task. Thus, agents that reduce GABAergic neurotransmission in the NBM by their inverse agonist action at the BZD receptor appear to enhance memory.

The purpose of the present experiment was to investigate the mnemonic effects of intra-NBM injections of the BZD partial inverse agonist, *N*-methyl-β-carboline-3-carboxamide (FG 7142) (for review see [14]). Memory was tested using the double Y-maze [21]; this paradigm provides an assessment of both reference and working memory on every trial, making it possible to identify treatment effects that are specific to memory rather than non-mnemonic variables such as sensory/perceptual capacity, motor activity or motivation. We have shown previously that intra-NBM injections of the GABA agonists, muscimol and baclofen, dose-dependently impaired memory [2,9]. As the mnemonic effects of intra-NBM injections of FG 7142 were unknown, a single dose of muscimol was included in the present study to verify that intracerebral injections would influence memory in the present paradigm.

2. Materials and methods

Treatment of the rats in the present study was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University policies and was approved by the Queen's University Animal Care Committee.

2.1. Subjects

Twenty male Sprague–Dawley rats, purchased from Charles River, Canada and weighing 200–225 g at the time of arrival were group-housed in hanging wire cages in a temperature-controlled (21 ± 1°C) colony room maintained on a 12-h light/dark cycle (lights on at 07.00 h). Water was freely available in the home cage. Food was rationed daily to maintain the rats at 80–85% of their free-feeding weights, adjusted for growth.

2.2. Surgery

Surgery was performed one week after arrival prior to training. The animals were handled daily before surgery. Rats were anaesthetized with sodium pentobarbital (Somnatol, 65 mg/kg, i.p.) and implanted bilaterally with chronic indwelling guide cannulae (0.64 mm diameter) aimed at a site 1.0 mm dorsal to the NBM. With the incisor bar set at 3.3 mm below the horizontal plane passing through the interaural line [28], the coordinates were 1.8 mm posterior to bregma, 2.6 mm lateral to the midline and 6.8 mm ventral to the surface of the skull. The cannulae were anchored to the skull with stainless steel screws and dental acrylic cement. Between injections, the guide cannulae were occluded with stainless steel wire pins (0.31 mm diameter).

2.3. Central injections

Two Hamilton microsyringes (10.0 μl) mounted in an infusion pump (Sage Instruments, Model 355) were used to infuse the drugs at a constant rate of 0.5 μl/30 s. The bilateral injections were performed simultaneously. The volume of all injections, including vehicle, was 0.5 μl. Injection cannulae, made of stainless steel tubing (0.31 mm diameter), were cut to extend 1.0 mm beyond the tips of the guide cannulae and were attached to the microsyringes by polyethylene tubing. To promote diffusion of the drug, the injection cannulae were kept in position for an additional 30 s following infusion.

2.4. Drugs

N-Methyl-β-carboline-3-carboxamide (FG 7142), 2-hydroxypropyl-β-cyclodextrin (cyclodextrin) and muscimol hydrobromide (Re-

search Biochemicals) were dissolved in physiological saline. As FG 7142 is not soluble in water, it was complexed with cyclodextrin, which increased solubility. The proportion of FG 7142 to cyclodextrin was 0.87–99.13%. Cyclodextrin has been shown to be a non-toxic solubilizer [29]. All solutions were prepared and frozen for later use.

2.5. Apparatus

The double Y-maze was elevated 76 cm above the floor. The center stem of the maze was 55 cm long and 15 cm wide and each arm, also 15 cm wide, extended 35 cm from the stem at an angle of 120°. Removable wooden barriers could be inserted at the end of each arm and in the middle of the stem to provide 15 cm^2 compartments. The floor consisted of stainless steel bars spaced approximately 1 cm apart, except at the junctions of the three arms, where the floor consisted of a triangular piece of Plexiglas. The maze walls (26 cm high) and barriers were painted light grey. Plastic food containers were placed in the center of the end wall of the goal and start boxes of each arm and in the center of the (removable) end wall of the stem. Froot Loops cereal was used as the reward and pieces were scattered under the grid floor to mask possible odor cues. Testing was carried out in a small, dimly lit room in which several visual cues (e.g., experimenter, light fixtures, door frame) were within sight of the maze.

2.6. Procedure

2.6.1. General training

Food deprivation began 7 days before the beginning of training, after the surgeries were performed. For several days prior to testing, the rats were handled daily and given Froot Loops in their home cages. Three days before training, the rats were placed in the maze for 10-min with barriers removed and Froot Loops in all food containers.

Training sessions were conducted at approximately the same time each day, one session per day, 7 days a week. Each session began with a fixed trial, i.e., when access to the second 'Y' was given, one of the arms was blocked. From session to session, the blocked arm was determined randomly such that half the sessions began with the right arm blocked and half with the left arm blocked. This determined the alternation sequence for subsequent trials. The rats received an additional 24 trials per session, with intertrial intervals being the time required to remove the rat from the goal box and place it into the start box of the first 'Y', nominally 0 s. Each trial began by placing the rat in one of the start boxes of the first 'Y'. The barrier was then removed and the rat was rewarded for going down the stem, the distal end of which was blocked by a removable barrier. Upon entering the region located in the middle of the stem, a barrier was dropped into place behind the rat, preventing re-entry into the first 'Y'. The barrier to the second 'Y' then was removed and the rat was rewarded for entering the appropriate goal box of the second 'Y'.

The correct choices required the use of both working and reference memory. The reference memory component was to always traverse the stem, regardless of which end arm of the first 'Y' was the starting position. The correct working memory choice was to enter the arm of the second 'Y' opposite to the one previously entered (i.e., simple alternation). If a reference memory error was committed, the trial was terminated and a forced trial (i.e., one of the arms of the second 'Y' was blocked with a barrier) was given to re-establish correct performance. The forced trial was not scored. An arm choice was defined to have taken place when the hind legs crossed onto the grid floor of the arm.

In the reference memory component, entries into the opposite arm of the first Y-maze instead of the stem were scored as reference memory errors. In the working memory component, entries into the arm most recently visited were scored as working memory errors. The number of errors in both components was recorded daily for all trials.

Training continued at 24 trials per session until choice accuracy in both components reached a criterion of at least 91% (22 of 24 choices correct) for three consecutive sessions.

2.6.2. Drug testing

The rats were tested as in training, except that delay intervals of 0, 30 and 60 s were introduced. For each trial, the delay was instituted by confining the rat to the start box of the first 'Y' before the reference memory choice. For each 24-trial session, the rat was given eight trials at each of the delay intervals, in random order. Each rat received six treatments – no injection, vehicle (345 μg cyclodextrin/0.5 μl saline per side), 0.2, 2.0 and 3.0 μg/0.5 μl FG 7142 per side and 0.1 μg/0.5 μl muscimol per side – the order varying between subjects. The rats were retrained without delays until criterion performance was achieved for two consecutive sessions following each treatment, prior to the next treatment.

2.7. Histology

After the completion of behavioural testing, the rats were injected with a lethal dose of sodium pentobarbitol and sacrificed by decapitation; the brains were extracted and placed in a 4% formalin/sucrose solution. They were stored in the solution for 7 days before being frozen and sliced into 70 μm coronal sections. The sections were mounted on glass slides and stained with thionin, after which they were classified as 'hits' (in the NBM) or 'misses' (outside the NBM) by an observer who was unaware of the performance of the animals.

3. Results

3.1. Acquisition

The mean ± standard error of the mean (S.E.M.) number of sessions to acquisition was 4.5 (±0.5) in the reference memory component and 4.7 (±0.5) in the working memory component. A paired t-test revealed that the two were not significantly different, $t_8 = -0.29$, $P > 0.05$.

3.2. Criterion sessions

As described earlier, animals were retrained following a treatment session until criterion was achieved again. The minimum number of sessions to criterion was two and no animal required more than four sessions following any treatment.

3.3. Histology

Of the original 20 rats, five failed to complete testing when their cannulae mounts were lost. Data from these rats were not included in subsequent analyses.

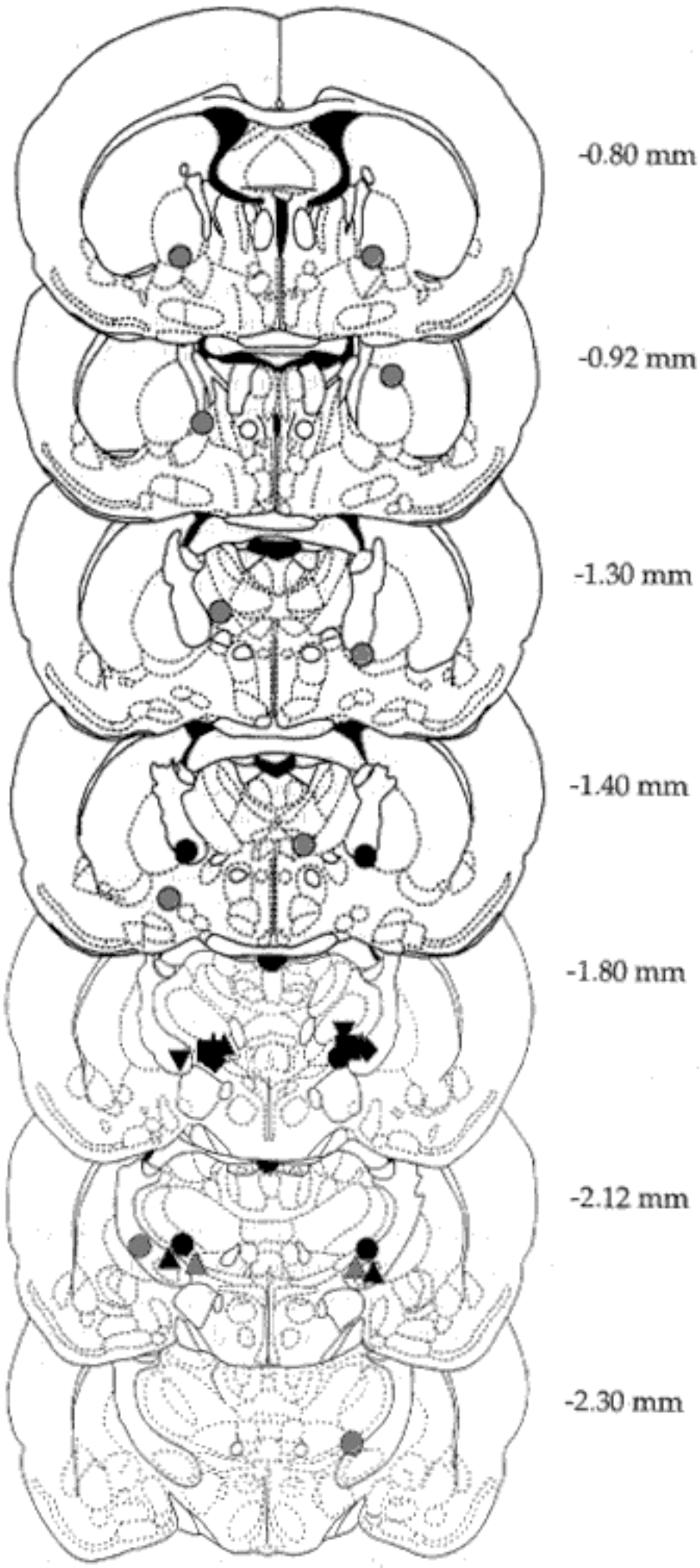

Fig. 1. Cannulae placements. Solid symbols represent 'hits' and stippled symbols represent 'misses'. Where more than one rat is shown on a section, different symbols are used for each rat. Numbers beside each section indicate the distance anterior or posterior to bregma, according to Paxinos and Watson [28].

The locations of the cannulae tips of the remaining 15 animals, all having completed the behavioural testing, are shown in Fig. 1; nine were classified as 'hits' and six as 'misses'. All behavioural analyses were based on the nine animals classified as 'hits'.

3.4. Drug testing

The number of correct choices at each delay for each memory component for each session was converted to a percentage of the total number of trials. For the no-injection condition at the 0-, 30- and 60-s delay, the mean (±S.E.M.) percent correct working memory choices were 88.3 (±4.07), 66.3 (±2.83) and 59.1 (±2.77), respectively; the corresponding values for the vehicle condition were 91.7 (±2.95), 75.6 (±4.60) and 62.5 (±3.02), respectively. Analysis of variance (ANOVA) of the working memory component revealed no significant treatment (no-injection vs. vehicle) effect, $F_{1,8} = 3.11$, $P > 0.05$, and no significant interaction of delay and treatment, $F_{1,8} = 0.65$, $P > 0.05$. As expected, the delay effect was significant, $F_{2,16} = 24.27$, $P < 0.05$. Similarly, for reference memory, respective means (±S.E.M.) were 97.2 (±1.84), 98.6 (±1.39) and 98.6 (±1.39) for no-injection and 98.6 (±1.39), 95.8 (±2.08) and 95.8 (±2.08) for vehicle. ANOVA revealed no significant effects. Thus, the data from the no-injection condition were dropped from subsequent analyses, as the vehicle condition was thought to be a better control.

With the no-injection data excluded, there were 15 scores (i.e., three delays for each of five treatments) for each rat for each memory type. As can be seen in Fig. 2, all treatments appeared to produce a large delay-dependent decrement in performance in the working memory component, but not in the reference memory component. FG 7142 doses of 0.2 and 2.0 μg appeared to improve working memory relative to the vehicle condition, especially at the 60-s delay, while muscimol caused an impairment, especially at the longer delays. In the reference memory component, performance following intra-NBM injections of all doses of FG 7142 did not appear to differ from performance following vehicle injection. Muscimol, on the other hand, appeared to impair reference memory at the 60-s delay.

This description of the data was supported by statistical analyses. Repeated measures two-way ANOVAs, using Greenhouse-Geisser adjusted degrees of freedom, were performed. Unadjusted degrees of freedom are shown below for clarity. Analysis of the percent correct working memory choices revealed a significant overall treatment effect, $F_{4,32} = 19.22$, $P < 0.01$, a significant delay effect, $F_{2,16} = 47.62$, $P < 0.01$ and a significant treatment by delay interaction, $F_{8,64} = 2.70$, $P < 0.05$. Tests of simple main effects on the interaction revealed a significant treatment effect at the 0-, 30- and 60-s delays, $F_{4,32} = 3.43$, $P < 0.05$, $F_{4,32} = 10.32$, $P < 0.05$, $F_{4,32} = 11.9$, $P < 0.05$, respectively. Dunnett's post hoc tests comparing each treatment to vehicle

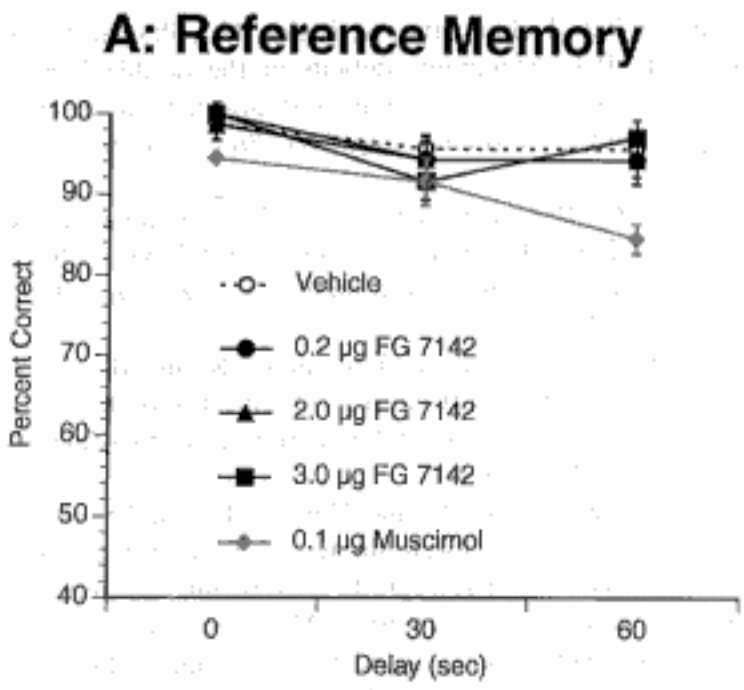

Fig. 2. Mean (±S.E.M.) percent correct reference (A) and working (B) memory choices. Intra-NBM injections of FG 7142 doses of 0.2 and 2.0 mg in 0.5 µl enhanced working memory at the 60-s delay. Muscimol in a dose of 0.1 mg in 0.5 µl impaired both reference and working memory.

separately at each delay revealed that the muscimol treatment impaired performance at all delays. FG 7142 doses of 0.2 and 2.0 µg significantly improved working memory at the 60-s delay.

The percent of correct reference memory responses showed a significant treatment effect, $F_{4,32} = 3.47$, $P < 0.05$ and delay effect, $F_{2,16} = 10.54$, $P < 0.05$. The interaction was not significant, $F_{8,64} = 1.05$, $P > 0.05$. Dunnett's post-hoc tests revealed that muscimol produced significantly more reference memory errors than vehicle.

4. Discussion

The present results can be summarized as follows. Although animals acquired each component of the double Y-maze task at a similar rate, the insertion of delays between trials during test sessions differentially affected the reference and working memory components. Intra-NBM injections of the benzodiazepine partial inverse agonist, FG 7142 dose-dependently enhanced working memory at the longest (60 s) delay. Unlike FG 7142, which reduces GABA-mediated neural inhibition, the $GABA_A$ receptor agonist muscimol impaired memory.

Although reference memory was not enhanced by FG 7142, it should be noted that baseline performance on this component of the task was highly accurate leaving little room for improvement. Thus, whether or not intra-NBM injections of FG 7142 can lead to an enhancement of reference memory remains an open question.

The double Y-maze task originally was developed in our laboratory as a procedure for assessing memory that contained an internal control for possible non-mnemonic effects (e.g., sensory-perceptual, motoric, motivational) of lesions or pharmacological treatments [21]. Thus, the non-mnemonic demands of the first and second choice in the double Y-maze were identical, the two differing only in their demands on recent or working memory. The differential mnemonic demands of the two components were validated by the observation that the insertion of delays impaired working, but not reference memory [4,9], a finding that was replicated here.

The present version of the double Y-maze task differed from the original version by using a single alternation as the working memory component. This procedure has been employed in one previous study in our laboratory (unpublished findings). The advantages of this procedure are that acquisition of criterion levels of performance is achieved more rapidly and that the rate of acquisition of the reference and working memory components is similar. This latter finding reduces the possibility that the differential effects of various treatments on working and reference memory can be attributed to differential difficulty of the two components, as we have discussed elsewhere [2,9,21].

One dose (0.1 µg) of the $GABA_A$ agonist muscimol was included in the present study as a standard for showing that intra-NBM injections affect memory, since the effects of FG 7142 were unknown. Muscimol impaired both working and reference memory. In two previous studies from this laboratory, 0.1 µg of muscimol into the NBM selectively impaired working memory [2,9]. However, delays were not included in the experiments involving muscimol in either of these papers. In the present study, the effects of muscimol at the 0-s delay appeared to be larger for working memory, replicating previous findings. Furthermore, at the 30-s delay, muscimol produced a large effect on working memory, with a minimal effect on reference memory. Thus, as shown previously shown [2,9,11,22,27],

intra-NBM injections of muscimol or GABA itself produced a relatively greater impairment of working memory.

This is the first study to report that intra-NBM injections of the benzodiazepine partial inverse agonist FG 7142 enhance memory. Previously, Mayo et al. [23] showed that the benzodiazepine inverse agonist β-CCM, when injected into the NBM prior to or immediately following exploration of two arms of a Y-maze, led to enhanced exploration of the novel third arm, in a subsequent task. The authors concluded that intra-NBM β-CCM improved cognitive processes. To the extent that their task involved memory for the arms visited during the first exposure to the Y-maze, the results of Mayo et al. [23] are in agreement with the present findings.

Cholinergic cells of the NBM are inhibited by GABA-ergic afferents and interneurons [15,25,40]. Sarter et al. [31] have suggested that the mnemonic function of NBM cholinergic neurons may be enhanced by treatments that reduce this GABA-ergic inhibition, while preserving the normal pattern of activity in these cholinergic neurons. Although it remains to be established that local injections of FG 7142 enhance the activity of NBM cholinergic cells, previous research has shown that systemic injections of another benzodiazepine partial inverse agonist, ZK 93 426 do enhance cortical acetylcholine release assessed using in vivo microdialysis [26]. It is possible that tonic activity of basal forebrain cholinergic neurons increased with higher doses of FG 7142, thereby weakening endogenous signals in these neurons (M. Sarter, personal communication). Consistent with this notion is the present finding that the highest dose of FG 7142 (3.0 μg) did not enhance working memory. Thus, the present results are consistent with the suggestion of Sarter et al. [31] that benzodiazepine partial inverse agonists may enhance memory and encourage further investigation of the mnemonic function of afferents to NBM cholinergic neurons.

Acknowledgements

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