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Localized injections of various compounds effecting neurotransmitter activity in the mammillary complex enhance (T-maze) avoidance retention

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Abstract

The mammillary complex is implicated in the amnesic syndrome associated clinically with Korsakoff's syndrome, Alzheimer's disease and experimentally with lesions in animals. There is however no direct evidence that the mammillary bodies are involved in long term memory processing. Mice were partially trained on a footshock avoidance task. Immediately after training drugs were injected into the mammillary complex. Retention was tested 1 week later by continuing training until each mouse made five avoidance responses in six trials. The results indicated that muscarine, nicotine, dopamine, glutamine and adrenoceptor agonists as well as GABA and 5-HT receptor antagonists and neuropeptide Y improved retention test performance relative to the control. Injection of the same drugs 1 mm above the injection site for the mammillary complex failed to significantly improve retention test performance. It is concluded that the mammillary complex, with its important connections to other areas of the limbic system, is involved in memory processing events that occur shortly after training.

Keywords: Mammillary complex; Memory; Retention; Acetylcholine; Dopamine; Norepinephrine; NMDA (*N*-methyl-D-asparate); Nicotine; Neuropeptide Y; GABA (γ-aminobutyric acid); (Mouse)

1. Introduction

Studies in patients with Korsakoff's syndrome, Alzheimer's disease and lesion studies in animals suggest the mammillary complex may be important for normal cognitive functioning. Lesions in the mammillary complex consistently occur in clinical and experimental models of Korsakoff's syndrome. Victor et al. (1971) found that 100% of patients with Korsakoff's syndrome had damage to the medial mammillary body. Others have also found consistent loss of mammillary complex nerve cells following chronic alcohol abuse (Mayes et al., 1988). Magnetic resonance imaging of amnestic patients revealed grossly reduced mammillary body nuclei in Korsakoff patients and some reduction in mammillary complex nuclei among non-Korsakoff amnesics (Squire et al., 1990). In another magnetic resonance imaging study, mammillary complex neurons were reduced in size in subjects over the age of 65 years as compared to the tectum control area (Raz et al., 1992). The mammillary body was also found in 95% of 25 patients with confirmed Alzheimer's disease to have either senile plaques and or neurofibrillary tangles (Grossi et al., 1989).

Evidence from animal studies involving either experimental lesions or chronic alcohol treatment suggests that the mammillary complex may be involved in learning. Irle and Markowitsch (1983) treated rats for 20 months with either pyrithiamine, to induce thiamine deficiency, or 30% alcohol solution. Both groups had either damage or loss of mammillary complex neurons which was associated with impaired acquisition of footshock avoidance in a shuttle box and of acquisition in a spatial discrimination task. A similar study found progressive mammillary complex cell loss was associated with the length of alcohol ingestion which was accompanied by impaired learning (Tako et al., 1991).

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The most abundant literature on the possible role of the mammillary complex in cognitive behavior is based on lesion studies. Early studies reported negative results, failing to demonstrate either impaired learning or retention in animals with mammillary body lesions (Kim and Chang, 1967; Thompson and Hawkins, 1961). Recently the majority of work suggests that learning and short term memory is impaired in animals following mammillary body lesions. Following electrolytic or chemical lesions in the mammillary complex, rats were found to have impaired learning for delayed nonmatching to place in an eight arm radial maze (Beracochea et al., 1989), and impaired spatial memory in a cross maze, and T-maze (Beatty et al., 1984; Rosenstock et al., 1977; Saravis et al., 1990). Similar lesions in cats induced impaired spatial working memory for alternation in a T-maze (Irle and Markowitsch, 1982). Rats with lesions of the mammillary complex are impaired in acquisition of a differential low rate of reinforcement task requiring accurate judgement of time where the subject must delay responding in order to receive reinforcement. This suggests the mammillary complex is involved in temporal information processing (Tonkiss and Rawlins, 1992).

To determine if the mammillary complex is involved in post-training memory processing and to facilitate further study of this brain region with others involved in memory 'consolidation', we investigated the neuropharmacology of the mammillary complex on retention of weak footshock avoidance training. Previous and current studies conducted in our laboratory demonstrate facilitation of retention following localized injections into other limbic system structures of drugs affecting receptor activity of classical neurotransmitters as well as neuropeptides (Flood et al., 1989, 1990 and unpublished studies). In the present study, a battery of compounds, affecting classical neurotransmitter activity, was injected into the mammillary complex immediately after training so they could not directly alter acquisition (i.e., learning). Long term retention for T-maze footshock avoidance training was tested 1 week later so that proactive effects of these short acting drugs would not directly affect retention test performance.

2. Materials and methods

2.1. Subjects

After at least 2 weeks in the laboratory, CD-1 male mice obtained from Charles River Breeding Laboratories, Wilmington, MA at 6 weeks of age, were caged individually 48 h prior to training and remained singly housed until retention was tested 1 week later. Animal rooms were on a 12 h light-dark cycle with lights going on at 06:00 h. Mice were assigned randomly to groups of 15 and were trained and tested between 08:00 and 14:00 h.

2.2. Apparatus and training and testing procedures

The T-maze and training procedures were described previously (Flood et al., 1992). The T-maze consisted of a black plastic alley with a start box at one end and two goal boxes at the other. The start box was separated from the alley by a plastic guillotine door which prevented movement down the alley until training began. An electrifiable stainless steel rod floor ran throughout the maze to deliver scrambled footshock.

Mice were not permitted to explore the maze prior to training. A block of training trials began when a mouse was placed into the start box. The guillotine door was raised and a buzzer sounded simultaneously; 5 s later footshock was applied. The goal box entered on the first trial was designated 'incorrect' and the footshock was continued until the mouse entered the other goal box, which in all subsequent trials was designated as 'correct' for the particular mouse. At the end of each trial, the mouse was removed to its home cage until the next trial. Four training trials were given at an intertrial interval of 30 s and the footshock intensity was 0.30 mA. The buzzer intensity was 55 db. Saline or drug solution was administered within 3 min after training into the mammillary complex. One week later, T-maze training was resumed until each mouse made five avoidance responses in six consecutive training trials. Trials to this criterion was taken as a measure of retention. Under this training condition, control subjects reliably show poor long term retention.

2.3. Drugs

Arecoline hydrobromide (1,2,5,6-tetrahydro-1-methyl-3-pyri-dinecarboxylic acid hydrobromide) a muscarinic receptor agonist, L-glutamic acid hydrochloride, a NMDA receptor agonist, and DMPP (1,1-dimethyl-4-phenylpiperazinium), a nicotinic receptor agonist, were obtained from Sigma Chemical Co., St. Louis MO. SKF-38393 ((+)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride) a dopamine D_1 receptor agonist, ketanserin tartrate (3-[2-[4-(4-fluorobenzoyl)-1-piperdinyl]-2,4(1*H*,3*H*)-quinazolinedione tartrate), a 5-HT receptor antagonist and bicuculline methiodide (6-(5,6,7,8-tetrahydro-6-methyl-1,3-dioxolo[4,5-*g*]-isoquindin-5-yl)-furo[3,4-*e*]-1,3-benzodioxo-8-(6*H*)-one methiodide), a GABA receptor antagonist), were purchased from Research Biochemical International, Natick, MA. Imidazoline nitrate, (ST587; 2-(2-chlor-5-trifluormethyl-phenyl-imino)-imidazolidin nitrate), a α_2-adrenoceptor agonist, was provided as a gift from Boehringer Ingelheim, Elmsford,

NY. Neuropeptide Y was obtained from Peninsula Laboratories, Belmont, CA. All drugs were dissolved in saline and coded to prevent experimenter bias. The range of drug doses tested (Fig. 1) were based on results obtained from concurrent studies conducted in other limbic system structures. To conserve on animals, the object was to determine the dose, if any, that reduced the mean trials to criterion to about seven on the retention test. Based on numerous such experiments, this is the optimal performance for mice on this task.

2.4. Drug administration

The surgical procedures used to prepare mice for localized injection of drug compounds were previously described (Flood et al., 1989,1990). In experiment 1, the injection coordinates for the mammillary complex were derived empirically as −3.4 mm anterior/posterior relative to bregma and 0.5 mm to the right of the central suture. The injection depth was 5.2 mm relative to the skull surface and injected at 3 degrees toward the midline. Fig. 1 shows a typical injection into the mammillary body. In brief, mice were anesthetized with methoxyflurane and placed in a stereotaxic instrument. The scalp was deflected and a hole drilled through the skull on the right side of the central suture. Mice were trained 48 h after surgery. Immediately *after* training mice were again placed in the stereotaxic under light enflurane anesthesia. Within 3 min *after* training, a 0.5 μl solution of saline or drug solution was injected into the mammillary complex over 60 s through a 30 gauge blunt stainless steel hypodermic tubing (Small Parts, Miami, FL) attached to a 10 μl syringe with PE-10 tubing and driven by a Sage Syringe Pump (Model 341A). The reliability of the injections was determined histologically by locating the tip of the needle tract in frozen brain sections following the retention test. The site of the injection was confirmed using a mouse forebrain stereotaxic atlas (Slotnick and Leonard, 1975). Ninety-three percent of the needle tracks terminated in either the supramammillary, lateral mammillary, or medial mammillary nuclei with 65% of the injections into the lateral or medial mammillary nuclei. Data from those receiving injections into the medial and rostral portions of the substantia nigra and ventral tegmentum were excluded. In experiment 2, we determined if diffusion along the needle tact could result in improved retention by injecting solution at a depth of 4.2 mm or 1 mm above the cite of the mammillary complex injections. Other coordinates were as stated above. The injections were in the posterior hypothalamic nucleus between the habenulo-interpeduncular and mammillo-tegmental tracts.

2.5. Statistical treatment of the data

In experiment 1, significance of the overall effect for each drug treatment was determined by a one-way analysis of variance (ANOVA) run on the trials to criterion from the retention test. Dunnett's *t*-test was used to determine which drug doses significantly reduced the mean trials to criterion relative to the mean of the saline control (Keppel and Zedeck, 1989; Winer, 1971). In experiment 2, an ANOVA run on trials to criterion and followed by Dunnett's *t*-test, was used to determine which drug significantly reduced the mean trials to criterion relative to the saline control group. The results of the retention testing are expressed as means and S.E.M.

2.6. Experiment 1

To test if the mammillary complex is involved in post-training memory processing and to determine which classical neurotransmitters might be involved, we

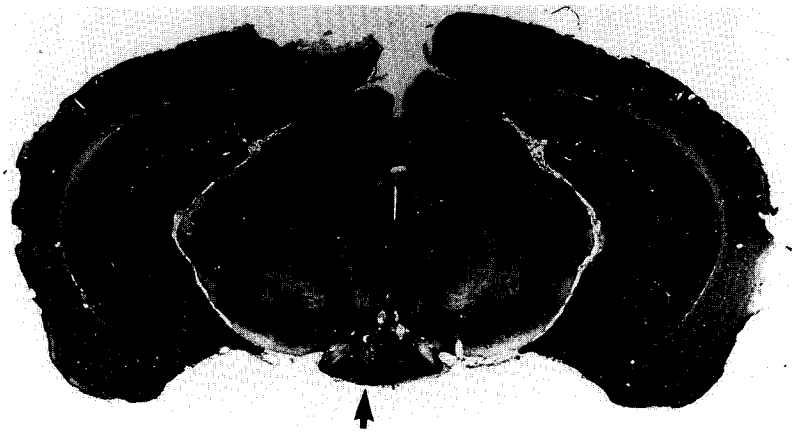

Fig. 1. The arrow indicates the site of injection into the mammillary body with a portion of the needle tract visible.

administered saline or drug solution into the mammillary complex area immediately after T-maze footshock avoidance training. Neuropeptide Y was included as previous research indicated that it affected retention in other limbic system structures but whether it improved or impaired retention was dependent on the site of the injection (Flood et al., 1989). The doses of the compounds tested (Fig. 1) were based on previous and concurrent studies with the same drugs in other limbic system structures. The range of doses tested did not always result in a fully developed U-shaped dose-response curve, but in each case sufficient doses were tested to establish a dose-dependent effect on retention and to obtain a mean trials to criterion on the retention test of about seven trials which is the optimal performance on this task, even when given more extensive training (Flood et al., 1990). Retention was tested 1 week after training and drug administration.

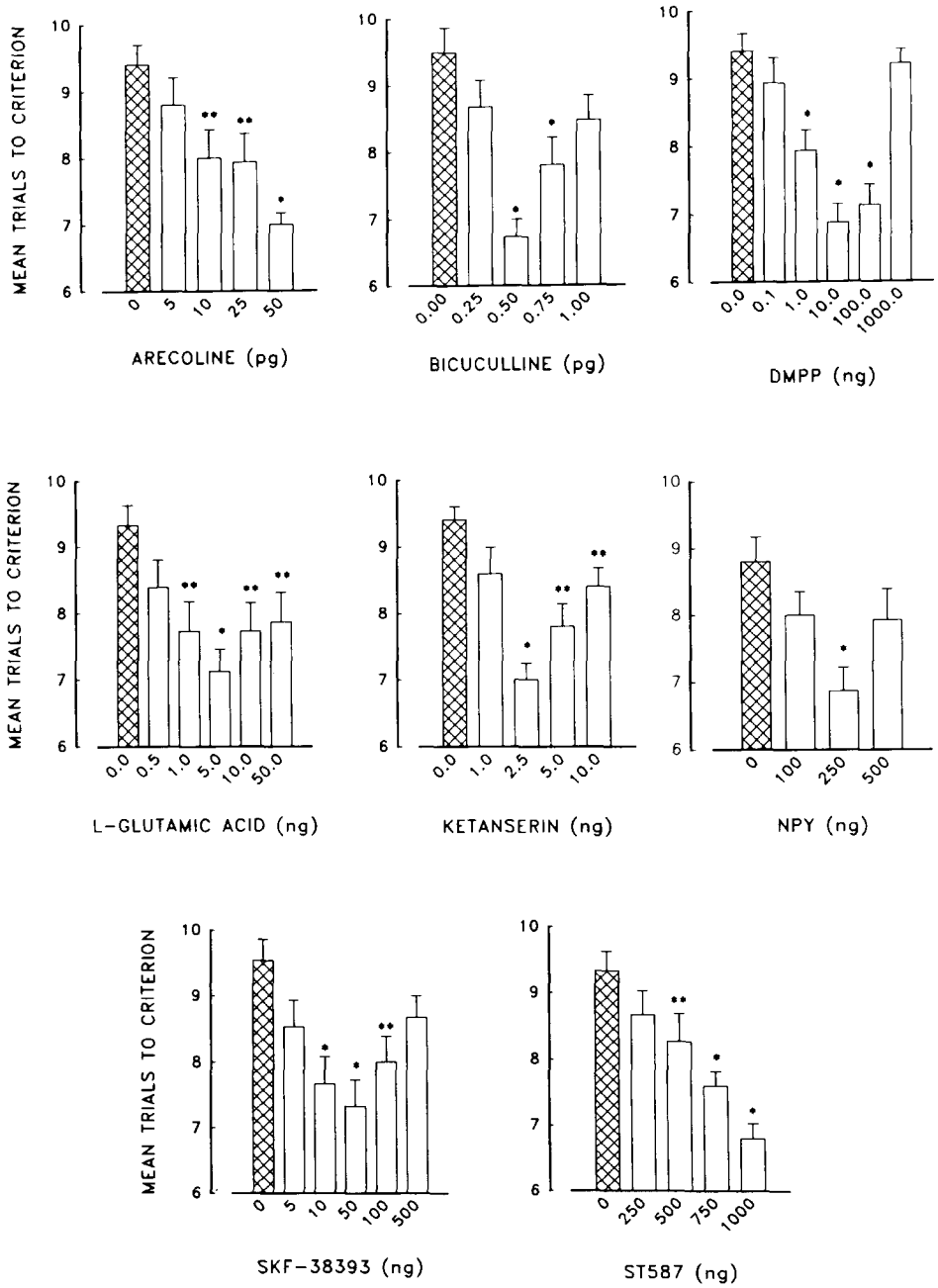

Fig. 2. Dose-dependent improvement of mean (+S.E.) trials to criterion on the retention by drugs effecting postsynaptic receptor activity of classical neurotransmitters and neuropeptide Y. Separate one-way ANOVA's run on mean trials to criterion yielded the following F values: arecoline, $F(4,70) = 6.82$, $P < 0.001$; bicuculline, $F(4,70) = 8.156$, $P < 0.001$; DMPP, $F(5,84) = 14.8$, $P < 0.001$; L-glutamic acid, $F(5,84) = 3.76$, $P < 0.005$; ketanserin, $F(4,70) = 9.96$, $P < 0.001$; neuropeptide Y (NPY), $F(3,56) = 4.44$, $P < 0.01$; SKF-38393, $F(5,84) = 4.66$, $P < 0.05$; ST587, $F(4,70) = 10.026$, $P < 0.001$).

2.7. Experiment 2

To address the possibility that improvement in memory retention following drug administration into the mammillary complex was due to diffusion of drug solution along the needle track into areas above the mammillary complex, the most effective dose of each drug that improved retention test performance in experiment 1 or saline was injected 1 mm above the mammillary complex injection site. The depth of the injection was raised from 5.2 to 4.2 mm relative to the skull surface. These injections terminated in the posterior hypothalamic nucleus. All other procedures were the same as in experiment 1.

3. Results

In experiment 1, all the compounds injected decreased the mean trials to criterion on the retention test in a dose-dependent manner (Fig. 2). Separate one-way ANOVA's run on mean trials to criterion indicated that arecoline, bicuculline, DMPP, ketanserin and ST587 yielded significant effects at $P < 0.001$. A similar analysis indicated a significant treatment effect at $P < 0.005$ for L-glutamic acid, $P < 0.01$ for neuropeptide Y and $P < 0.05$ for SKF-38393. The F values of the ANOVAs are given in the legend to Fig. 2. Fig. 2 also indicates the dose range over which each drug significantly decreased the mean trials to criterion relative to the control using Dunnett's t-test.

In experiment 2, a one-way ANOVA run on mean trials to criterion from the retention test detected no significant effect ($F < 1$) of the memory enhancing drug dose of experiment 1, injected 1 mm above the mammillary complex. The means and standard errors varied among the seven drug groups from a low of 8.73 ± 0.38 for 5 ng of L-glutamic acid to a high of 9.20 ± 0.32 for neuropeptide Y compared to a mean of 9.13 ± 0.40 for the saline control group.

4. Discussion

4.1. Summary of findings

While the importance of the mammillary complex in memory retention has been speculated on, the findings of these experiments indicate that manipulating postsynaptic activity with adrenoceptor agonists, acetylcholine, dopamine, glutamine and nicotine receptor agonists and GABA and 5-HT receptor antagonists as well as neuropeptide Y improved retention test performance in a dose-dependent manner. Since these compounds were administered after training and 1 week prior to testing retention, we interpret their effect on retention test performance as being the result of improvement of memory processing occurring shortly after training. Injecting these compounds 1 mm above the mammillary complex injection site failed to improve retention which indicated that there was a reasonable degree of localization of the memory enhancing effects in and close to the mammillary complex and supramammillary nucleus. Diffusion of 0.5 μl of a highly concentrated alizarin red dye solution did not diffuse out of the mammillary body proper over a 30 min period but did reach tuberomammillary nucleus below the mammillary complex. Dye diffusion from supramammillary nucleus injections only reached the rostral portion of the ventral tegmentum.

4.2. Receptors in the mammillary complex

The literature confirms that each of the transmitters targeted by the compounds administered have receptors in the mammillary nuclei. However, it has not been established for all the neurotransmitter receptors where the neurons are located. Several studies have reported dopamine terminals in the mammillary body which arise from the supramammillary region (Swanson, 1982). Studies indicate that all mammillary subnuclei are richly provided with GABAergic terminals (Gonzalo-Ruiz et al., 1992; Ottersen and Storm-Mathisen, 1984). Projections from the raphe medial and dorsal nuclei presumably are responsible for the 5-HT receptors (Meibach, 1984) as locus ceruleus projections are for adrenoceptors (Palacios and Wamsley, 1984). Glutamine is likely the neurotransmitter for the subicular projections to the mammillary complex and as well as endogenous glutamatergic neurons (Ottersen and Storm-Mathisen, 1984). The mammillary complex has a moderate number of muscarinic terminals but a high level of nicotinic terminals (Butcher and Woolf, 1984).

4.3. Conclusions

The mammillary complex is well connected to other limbic system structures implicated in those processes responsible for the conversion from working to reference memory. This study clearly indicates that post-training manipulations of these processes by drugs effecting synaptic activity of classical neurotransmitters and neuropeptide Y, enhanced long term retention. An erroneous impression that memory processing is under the control of a particular neurotransmitter has occurred because research has concentrated on a limited area. As most theories of learning and memory envision the use of complex multi-synaptic pathways, the view that memory processing is under the control of a single or select few transmitters is not reasonable. Each type of synapse offers an opportunity to modulate the process. This research shows the involvement

of acetylcholine (muscarinic and nicotinic), dopamine, norepinephrine, serotonin, GABA and neuropeptide Y in long term memory processing when injected into the mammillary complex. As the area has relatively high levels of opioids and hormones, it is likely that these will be able to modify 'consolidation'. With an expanded view of the potential therapeutic options, pharamcological means of alleviating impaired learning and memory may prove more productive in diseases where the mammillary complex may be involved such as Korsakoff's syndrome, Alzheimer's disease and Down's syndrome.

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