ELSEVIER

Pharmacology, Biochemistry and Behavior 72 (2002) 209–212

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Basal forebrain infusions impair delayed-non-match-to-sample radial arm maze performance

James J. Chrobak, T. Celeste Napier*,[1]

Department of Pharmacology and Experimental Therapeutics, Stritch School of Medicine, Loyola University Chicago, 2160 South First Avenue, Maywood, IL 60153, USA

Received 25 June 2001; received in revised form 23 October 2001; accepted 6 November 2001

Abstract

Direct site infusion of drugs into the brain is a powerful tool for examining the function of specific brain regions. While comparing the effects of various drugs injected into distinct regions of the basal forebrain on cognitive and motor endpoints, we observed that the ventral pallidum (VP) appeared to be sensitive to vehicle control infusions when cognitive indices were measured. To characterize this initial observation, the present study examined the effects of vehicle infusions into the VP on performance of a delayed-non-match-to-sample (DNMTS) radial arm maze (RAM) task. A within-subjects design was used. Male Sprague–Dawley rats were trained to perform this task with a 1-h delay imposed between the fourth and fifth arm selection. Following acquisition, animals were implanted with bilateral, indwelling cannulae positioned over the VP. Following surgery, maze performance was reestablished and rats were given one of five intra-VP treatments (two sham and three vehicle infusions) in counterbalanced order. Each rat received one treatment a week, on the third day of five consecutive testing days each week. Vehicle, but not sham, treatments produced deficits on the day of treatment and on two subsequent testing days. These findings demonstrate a persistent sensitivity of the VP to fluid perturbation and, when contrasted with the literature for other basal forebrain regions, it appears that this effect is unique. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Basal forebrain; Ventral pallidum; Radial arm maze; Memory; Rat

1. Introduction

The ventral pallidum (VP) contains cholinergic and GABAergic corticopetal neurons that are likely to play a crucial role in modulating cortical physiology (Zaborsky, 1992; Gritti et al., 1993). Thus, this projection can presumably influence a broad range of cognitive phenomenon including sensory processing, attention, and memory (Saper, 1987). Several strategies have been employed to examine the role of this ascending projection in cognition including neurotoxic lesions and electrical stimulation (Robbins et al., 1989; Kilgard and Merzenich, 1998). Local site infusion of drugs has also been used to examine the role of this system in cognitive function (Nagel and Huston, 1988; Holley et al., 1995). When combined with a chronic indwelling cannulae, this method allows for the application of different treatments, over repeated sessions, within a single subject.

Several studies have successfully utilized acute, local site infusions to assess the role of the medial septal nucleus in cognition (Bostock et al., 1988; Chrobak and Napier, 1992; Givens and Olton, 1990). Like the VP, the medial septum also contains cortically directed cholinergic and GABAergic neurons (Amaral and Kurz, 1985; Freund and Antal, 1988), but this projection selectively innervates the hippocampus. In attempting to complement behavioral studies of the medial septum with those of the VP, we observed that the VP was particularly sensitive to fluid perturbations following local site infusion of physiological saline. Following on this initial observation, we presently report that fluid perturbation alone produces deficits in the performance of a delayed-non-match-to-sample (DNMTS) radial arm maze (RAM) task that persist for several days following treatment.

* Corresponding author. Tel.: +1-708-216-3261; fax: +1-708-216-6594.

E-mail address: cnapier@luc.edu (T.C. Napier).

[1] Current address: Department of Psychology, University of Connecticut, Storrs, CT 06269, USA.

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(01)00752-3

2. Materials and methods

Male Sprague–Dawley rats ($N=22$; Charles Rivers Breeders, Wilmington, DE; 250–300 g), individually housed and maintained in a colony room with a 12-h light–dark cycle, were used. Beginning 5 days prior to behavioral training, daily food rations were limited in order to reduce the animals to 85% of their free-feeding weight. During preoperative training, rats were allowed to gain 5 g of body weight per week.

Rats were trained to perform a standard RAM task as previously described (Chrobak and Napier, 1992). Briefly, rats were allowed access to eight arms of a radial maze. Each arm was baited with a single 45-mg Noye's pellet. Rats were given a single daily trial in which they were allowed to collect all eight pellets and were removed from the maze if: (1) they collected all eight pellets; (2) they made a maximum of 20 errors defined as a reentry into an arm already entered previously during that day's trial, or (3) 5 min elapsed. Following acquisition (20 standard trials), the rats were trained to perform a DNMTS-RAM with a 1-h delay imposed between the fourth and fifth arm choices. During the predelay session, rats were placed on the maze and allowed to visit four predetermined arms (e.g., Bostock et al., 1988; Chrobak and Napier, 1992; Chrobak et al., 1989). The set of arms chosen for each daily session was randomly selected and baited with a single 45-mg Noye's pellet. Access to the alternate arms was prevented by a clear Plexiglas barrier placed at the entrance of four chosen arms. Following the delay, the rats were returned to the maze and allowed to choose among all eight arms. Only arms not visited during the predelay session contained food pellets. Thus, the rat was rewarded for nonmatching to the predelay sample set. Rats were allowed to choose until the four remaining baited arms were visited or until a total of 12 postdelay choices were made. The number of correct choices in the first four postdelay choices, the total number of postdelay errors, and the latency per arm choice served as dependent measures. Latency per arm choice was defined by the total time to complete the task divided by the total number of arm entries.

Following acquisition, rats were anesthetized with sodium pentobarbitol (50 mg/kg) and implanted with bilateral cannulae (26 gauge) aimed at the VP (AP 0.5 mm; ML +2.5 mm; DV 7.0 mm; toothbar was set at −0.3 mm). Cannulae were anchored to the skull using four stainless-steel screws embedded within the skull and secured with cranioplastic cement. Obturators (33 gauge), 1.0 mm shorter than the cannulae, were inserted into the guide cannulae during surgery to maintain patency during the course of behavioral testing.

Following recovery from surgery (1 week), a within-subjects design was used to examine the effects of sham and vehicle treatments DNMTS-RAM task performance. Behavioral assessments were made daily Monday to Friday, and all treatments were administered each Wednesday, with the treatment order randomized. The treatments included: (1) a sham treatment that involved light restraint and the insertion of an injector cannula into the guide cannula but not into tissue; (2) an injector treatment that included the insertion of the injector into the VP, but without fluid infusion; (3) infusion of 0.5 μl of sterile physiological saline (pH = 7.4); (4) infusion of 0.25 μl of sterile physiological saline; and (5) infusion of 0.25 μl of an artificial cerebrospinal fluid solution (CSF in mM: NaCl 150, KCl 2.9, $MgCl_2$ 7.8, HCO_3 35.9, and dextrose 2.2). Intracerebral infusions were administered bilaterally at the rate of 0.1 μl/min and the injectors were left in the cannulae for an additional minute after completing the infusion. All infusion and sham treatments, counterbalanced among animals, occurred immediately (within 5 min) after the completion of the predelay session. Following the infusion, rats were returned to their home cages and tested approximately 50–55 min later. At the end of the behavioral testing, rats were killed and injection locations were visually verified from 50-μm-thick brain sections stained with Cresyl violet. Injector tip placements indicated sites within the VP and the rostral aspect of the sublenticular substantia innominata for 18 rats [0.4–0.3 mm from the bregma and 2.5 ± 0.3 mm from the midline]. Four animals were excluded from the data analysis due to misplacement of the cannulae or significant tissue damage surrounding the cannulae location.

The data were analyzed for the remaining 18 rats using a repeated-measures analysis of variance (RMANOVA). A priori planned Dunnett's paired *t* tests were used to compare from vehicle infusions to injector only, and post hoc Tukey's HSD were used to compare responses across days. Data are presented as mean ± S.E.M.

3. Results

Performance measures for the entire group ($N=18$) during the Wednesday prior to treatments were 3.4 ± 0.3

Table 1
Effect of sham and vehicle treatments into the VP on DNMTS-RAM performance (1 h delay)

	Sham	Injector only	Saline (0.5 μl)	Saline (0.25 μl)	ACSF (0.25 μl)
Correct choices	3.3 ± 0.2	3.4 ± 0.2	2.8 ± 0.3 **	2.9 ± 0.3 **	2.9 ± 0.4 **
Postdelay errors	0.7 ± 0.4	0.8 ± 0.3	1.5 ± 0.4 **	1.7 ± 0.5 **	1.6 ± 0.4 **
Latency/choice (s)	7.0 ± 1.0	7.5 ± 1.1	7.1 ± 1.3	7.8 ± 1.0	7.2 ± 1.4

* $P<.05$ vs. injector-only treatment (Dunnett's *t* tests).
** $P<.025$ vs. injector-only treatment (Dunnett's *t* tests).

Table 2
Number of correct choices for each day during the week when either injector-only or saline treatment was administered into the VP

	Monday	Tuesday	Wednesday	Thursday	Friday
Injector only	3.5 ± 0.2	3.3 ± 0.1	3.4 ± 0.2	3.3 ± 0.2	3.4 ± 0.2
Saline (0.25 μl)	3.4 ± 0.2	3.3 ± 0.2	2.9 ± 0.3 **	2.8 ± 0.4 **	3.0 ± 0.4 *

Treatments administered only on Wednesdays, with order of treatments counterbalanced across animals.
* $P < .05$ vs. Tuesday performance (Tukey's HSD).
** $P < .25$ vs. Tuesday performance (Tukey's HSD).

correct choices, 0.8 ± 0.4 postdelay errors, and 8.4 ± 1.8 s/choice. This performance is comparable to performance for the days prior to cannulae implantation. Performance measures for the sham treatment versus injector-only treatment were not different, thus, for all subsequent statistical analyses, the injector-only condition served as the control. An overall RMANOVA for performance indices on the day of treatment indicated a significant treatment effect on the number of correct choices and number of postdelay errors [F's(3, 51) > 3.3, P's < .025]. Moreover, performance deficits occurred for both the number of correct choices [Dunnett's $t(17) = 3.19$, $P < .01$] and postdelay errors [Dunnett's $t(17) = 4.02$, $P < .01$] for all vehicle treatments on the day of treatment (Table 1). No change in the latency per choices was observed.

Performance indices for the 2 days following vehicle infusions were also impaired. RMANOVA of performance indices for each day of the week for trials during the week of the 0.25-μl infusion indicated a significant day effect on both correct choices and postdelay errors [$F(3,51) > 3.1$, P's < .05]. The number of correct choices decreased and the number of postdelay errors increased for all vehicle treatments [Dunnett's $t(17) > 2.9$, P's < .05]. Table 2 presents the number of correct choices for the injector-only and the low-volume saline (0.25 μl) condition. To assess for any chronic decrement in performance, we performed an RMANOVA for the number of correct choices and postdelay errors for each rat's performance on the day prior to treatment, independent of treatment order (for each consecutive Tuesday for 5 weeks; Table 3). No significant decrement in performance across weeks was observed [$F(3,51) < 2.21$, $P > .05$], although the daily performance of some individual rats appeared to be declining (data not shown). Eleven of the 18 rats were tested for three more weeks in order to examine the effects of additional vehicle infusions on task performance. An analysis of group performance on the Tuesday prior to treatment indicated that chronic performance decrements did develop on both the number of correct choices and postdelay errors [F's(2,30) > 4.89, P's < .025].

Table 3
Performance measures for all subjects each Tuesday (prior to any weekly treatment) independent of treatment order

	Week 1	Week 2	Week 3	Week 4	Week 5
Correct choices	3.3 ± 0.2	3.5 ± 0.2	3.3 ± 0.2	3.2 ± 0.4	3.1 ± 0.4
Postdelay errors	0.8 ± 0.3	0.7 ± 0.4	0.9 ± 0.4	0.9 ± 0.4	1.0 ± 0.5

4. Discussion

These data demonstrate that vehicle infusions into the VP produced a consistent impairment in the performance of the DNMTS-RAM task. This deficit appears to be related to the fluid infusion for it was not observed following either sham procedure performed, i.e., if no fluid was infused, there was no response difference between when injectors remained within the cannulae or extended beyond the cannulae tips and penetrated the VP. The vehicle-induced deficit lasted for a substantial time (the impairment persisted for at least 2 days), but was not permanent (animals recovered by 5 days).

These deficits contrast with the absence of a vehicle-induced deficit in rats performing a standard 8- or 12-arm radial maze paradigm without any delay inserted between choices (Robinson et al., 1988; Napier and Chrobak, 1992). A few studies have illustrated the sensitivity of basal forebrain regions to vehicle infusion in passive avoidance responding (Nagel and Huston, 1988) and have demonstrated a relationship of performance deficits to task difficulty (Elrod and Buccafusco, 1988). Research emphasis has been placed upon the forebrain cholinergic corticopetal system and its role in cognitive dysfunction (see Sarter and Bruno, 1997, for extensive review). The rostral extreme of the forebrain cholinerigc column includes neurons within the medial septal nucleus. We have used the present experimental protocol to assess performance following infusions into this nucleus without any deleterious effect (Chrobak and Napier, 1992; Chrobak et al., 1989). Moreover, such treatments within the VP do not alter motoric performance (Napier and Chrobak, 1992; and the present study). The convergence of these evidences allows for the speculations that (1) motor and cognitive tasks engage subsets of VP neurons, and (2) the cholinergic subset within the VP, which is engaged by more challenging mnemonic tasks, may be particularly sensitive to fluid perturbation. While further experimentation is needed to validate these possibilities, the present study clearly highlights the critical importance of sham and vehicle control groups in protocols that involve manipulations (especially those involving drug infusions) of the

basal forebrain. On this note, De Souza Silva et al. (2000) have demonstrated that both vehicle infusion or needle insertion alone is sufficient to induce alterations in cortical cholinergic parameters in both anesthetized and freely moving rats.

Acknowledgments

The authors thank Dowon An for her help in testing animals.

References

Amaral DG, Kurz J. An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation in the rat. J Comp Neurol 1985;240:37–59.

Bostock E, Gallagher M, King RA. Effects of opiod microinjections into the medial septal area on spatial memory in rats. Behav Neurosci 1988;102:643–52.

Chrobak JJ, Napier TC. Antagonism of GABAergic transmission within the septum disrupts working/episodic memory in the rat. Neuroscience 1992;47:833–41.

Chrobak JJ, Stackman RR, Walsh TJ. Intraseptal administration of muscimol produces dose-dependent memory impairments in the rat. Behav Neural Biol 1989;52:357–69.

De Souza Silva MA, Hasenohrl RU, Tomaz C, Schwarting RK, Huston JP. Differential modulation of frontal cortex acetylcholine by injection of substance P into the nucleus basalis magnocellularis region in the freely-moving vs. the anesthetized preparation. Synapse 2000;38:243–53.

Elrod K, Buccafusco JJ. Microinjection of vehicle into the nucleus basalis magnocellularis results in task-specific impairment of passive avoidance responding. Res Commun Psychol, Psychiatry Behav 1988;13:271–83.

Freund TF, Antal M. GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. Nature 1988;336:170–3.

Givens BS, Olton DS. Cholinergic and GABAergic modulation of medial septal area: effect on working memory. Behav Neurosci 1990;104:849–55.

Gritti I, Mainville L, Jones BE. Codistribution of GABA- with acetylcholine-synthesizing containing neurons in the basal forebrain of the rat. J Comp Neurol 1993;329:438–57.

Holley LA, Turchi J, Apple C, Sarter M. Dissociation between the attentional effects of infusions of a benzodiazepine receptor agonist and an inverse agonist into the basal forebrain. Psychopharmacology 1995;120:99–108.

Kilgard MP, Merzenich MM. Cortical map reorganization enabled by nucleus basalis activity. Science 1998;279:1714–8.

Nagel JA, Huston JP. Enhanced inhibitory avoidance learning produced by post-trial injections of substance P into the basal forebrain. Behav Neural Biol 1988;49:374–85.

Napier TC, Chrobak JJ. Evaluations of ventral pallidal dopamine receptor activation in behaving rats. NeuroReport 1992;7:609–11.

Robbins TW, Everitt BJ, Ryan CN, Marston MH, Jones GH, Page KJ. Comparative effects of ibotenic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. Behav Brain Res 1989;35:221–4.

Robinson SE, Hambrecht KL, Lyeth BG. Basal forebrain carbachol injection reduces cortical acetylcholine turnover and disrupts memory. Brain Res 1988;445:160–3.

Saper CB. Diffuse cortical projections systems: anatomical organization and role in cortical function. In: Mountcastle VB, Plum F, Geiger SR, editors. Handbook of physiology: Section I. The nervous system vol. 5, Part 1. Bethesda: American Physiological Society, 1987. pp. 169-210.

Sarter M, Bruno JP. Trans-synaptic stimulation of cortical acetylcholine and enhancement of attentional functions: a rational approach for the development of cognition enhancers. Behav Brain Res 1997;83:7–14.

Zaborsky L. Synaptic organization of basal forebrain cholinergic projection neurons. In: Levin ED, Decker MW, Butcher LL, editors. Neurotransmitter interactions and cognitive function. Boston: Birkhäuser, 1992. pp. 27–65.