EFFECTS OF NEUROTOXINS ON SOME BEHAVIORAL MANIFESTATIONS

Marie KOUPILOVÁ, Josef HERINK
Department of Toxicology, Purkyně Military Medical Academy, Hradec Králové

Summary

Behavioral tests were employed in the study of central effects of the selected neurotoxins mescaline, FAZ-4 and DSP-4 at stereotactical administration into three cerebral structures (ventriculus cerebri lateralis, ncl. basalis Meynerti and nc. septi medialis).

The results have confirmed the assumed negative action with cholinergic and monoaminergic dysfunctions, manifested by a disorder in the recall from animal memory immediately after their administration and with latency of several weeks. Different effects of individual neurotoxins on animal behaviour were observed also in dependence on the structure of administration.

KEY WORDS: Learning; Memory; Neurotoxins; Laboratory rats; Neurotransmitter's deficits; Intracerebral administration.

Selective destruction of relatively homogenous neuronal populations by means of specific neurotoxins represents an important experimental tool for modelling various pathological conditions of the central nervous system. A typical example of such an approach is the inducement of destruction of the central cholinergic system by means of ethylcholine mustard aziridinium (AF64A), which is generally considered to be an experimental model of degenerative changes leading to human dementia (14).

Aziridines are derivatives of the simple nitrogenous compound ethylene imine or aziridine. They act as strong alkylating reagents and consequently react with functional groups of biomolecules (9). N-(3,5-dimethoxy-4-propoxyphenylethyl) aziridine (FAZ-4) is one of the newly synthesized representatives of this group of substances according to an original US patent (15). From the viewpoint of its structure, FAZ-4 is a derivative of mescaline and some effects on the central monoaminergic system can thus be assumed (7). The present authors have already demonstrated an ability of FAZ-4 to increase the activity of acetylcholinesterase (AChE) in selected parts of the brain of the laboratory rat. As to intensity, this effectiveness is practically the same as in AF64A (5). FAZ-4 also possesses convulsive properties similar to those of pentylenetetrazole (6).

The present paper aims to test the effects of FAZ-4 and mescaline after intracerebral administration in three typical experimental tests of learning and memory. The effects of both compounds then compared with the effect of the identically administered model neurotoxin N-(2-chlorethyl)-N-ethyl-2--bromobenzylamine (DSP-4), which induces degeneration of noradrenergic neurotransmission (3).

Methods

Behavioral tests were carried out on the model of spatial orientation of animals in the T-maze with food motivation, on the model of aversively motivated behaviour of animals in the Y-maze, and operant behaviour in the Skinner box. The selected methods are routinely used in behaviour research and described in the literature (7, 4, 21).

Laboratory male rats of the Wistar strain weighing 220-250 g were used for the experiments. The animals were adapted to the conditions of the laboratory vivarium for a period of 14 days. Out of the experimental sessions, the animals were kept in plexiglass cages with three animals in each. During the experiments with appetitive motivation the diet of the animals was decreased to 8 g for a rat per day, water was accessible ad libitum. One group always consisted of 6 animals.

Administration of drugs was performed intracerebrally by means of a stereotactically introduced cannula into the following structures: ventriculus cerebri lateralis, ncl. basalis Meynerti and ncl. septi medialis in thiopental anaesthesia.

The coordinates of the intracerebral cannula are as follows ventriculus cerebri lateralis - AP = + 1, L = 1.6, V = 3.75; ncl. basalis Meynerti - AP = + 0.8, L = 2.6, V = 8.0; ncl. septi medialis - AP = - 0.75, L = 0, V = 5.5.

Mescaline was administered in a dose of 0.1 mg in a volume of 5 μl of NaCl, 0.9 %. Its effect was investigated in intervals of 30, 60 and 120 min after administration and further in a period of 1-22 days of action on spatial behaviour of animals in the T--maze. In the test of operant behaviour, mescaline was administered in a dose of 0.3 and 0.5 mg intracerebroventricularly and its effects were investigated after 24 hours and then daily until the effect disappeared.

FAZ-4 was administered in a dose of 0.3 mg in 3 μl of NaCl, 0.9 %. Its effects on spatial orientation of animals in the T-maze were investigated 30 and 120 min after administration and further daily for a period of three weeks.

In the test of operant behaviour, FAZ-4 was administered in a dose of 0.3 mg in 3 μl of NaCl, 0.9 %. The tests were performed under control conditions prior to the introduction of cannulae, after the introduction of cannulae, and after the administration of the drug.

DSP-4 was administered in a dose of 1.0 mg in a volume of 3 μl of NaCl, 0.9 %. Its effects were investigated 30 and 90 min after administration and further nine days after its administration in the experiment with appetitive motivation in the T-maze aversive motivation in the Y-maze.

In the T-maze, time (sec) needed to reach the goal, in the Y-maze, time of escaping a painful stimulus, and in the Skinner box, time of approach to the service device of the food-house were measured.

Statistical evaluation of results was carried out by analyzing the dispersion and the significance of the difference was evaluated by a t-test with Bonfferoni's correction of BMDP programme P7D.

Results

Mescaline, administered in an i.c. single dose of 0.1 $mg.kg^{-1}$ in a volume of 5 μl of NaCl, 0.9 %, prolonged latency time of finding the target in the T-maze ($P < 0.001$) on day 0 of the experiment over 60 and 120 min after the administration of the drug in those animals which received it into the ncl. basalis Meynerti and the ncl. septi medialis. A significant difference ($P < 0.01$) in comparison with the control group was also found in the animals whom this drug was administered into the lateral cerebral ventricle. Depending on time, deterioration of spatial orientation was observed in these animals after 120 min and on days 1 and 2

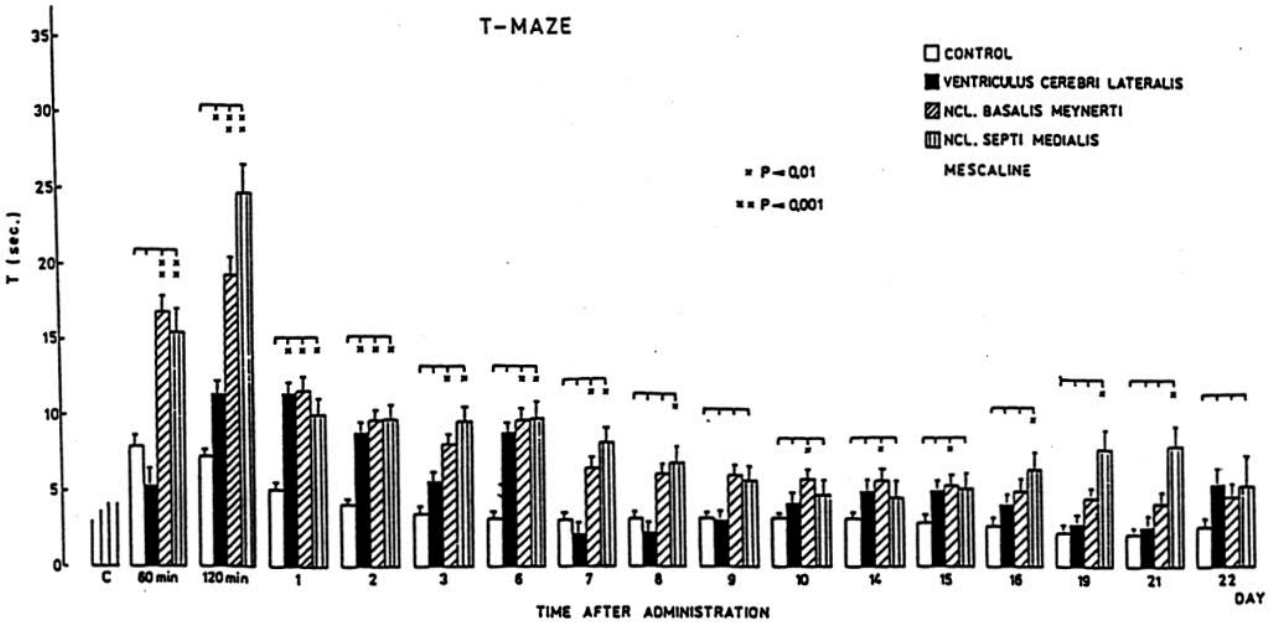

Fig. 1 Effect of mescaline in a dose of 0.1 mg i.c. on spatial orientation of animals tested in the T-maze

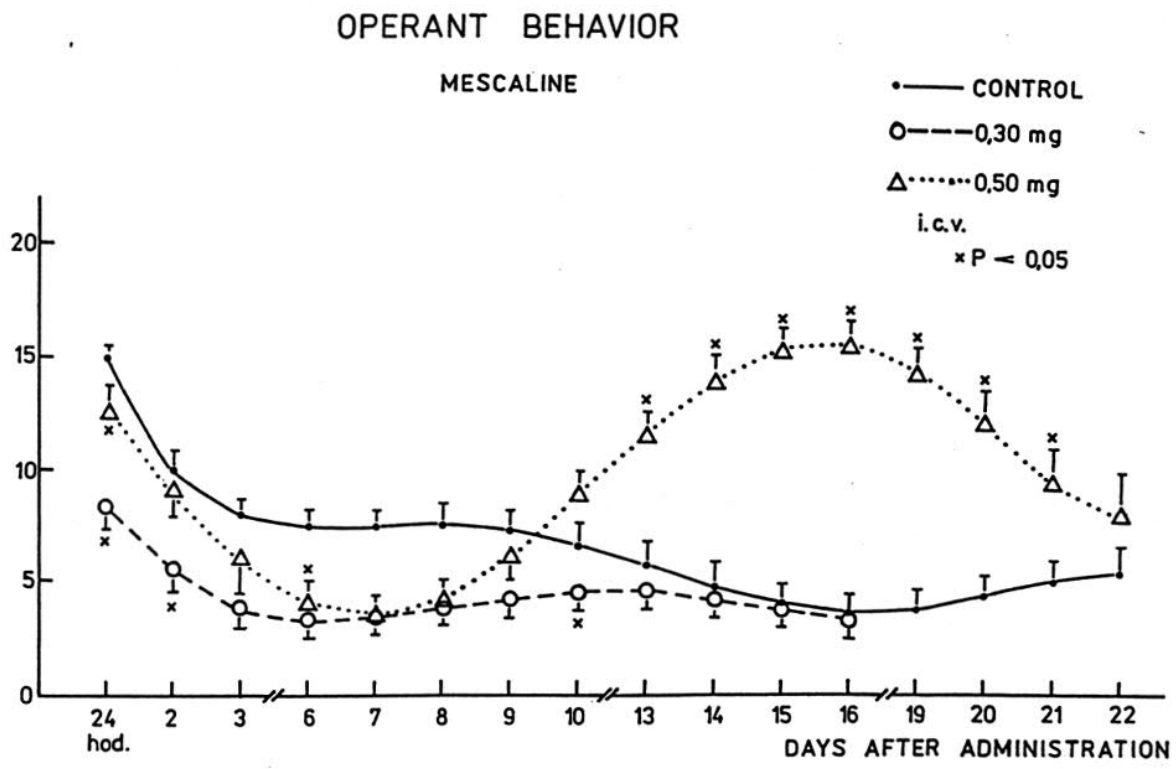

Fig. 2 Effects of mescaline administered in doses of 0.3 and 0.5 mg i.c.v. on operant behaviour of animals in the Skinner's box

after mescaline administration. A decrease of latency time ($P < 0.01$) lasted for the longest period in the animals whom the drug was administered into the NBM and the medial septum. In both groups, a two-stage effect of drug action was observed (Fig. 1).

After intracerebroventricular administration of mescaline, changes in operant behaviour were also observed (Fig. 2). A higher mescaline dose of 0.5 mg prolonged latency time of the accession of the animal to the service device of the food-house including saccharose consumption in a period of

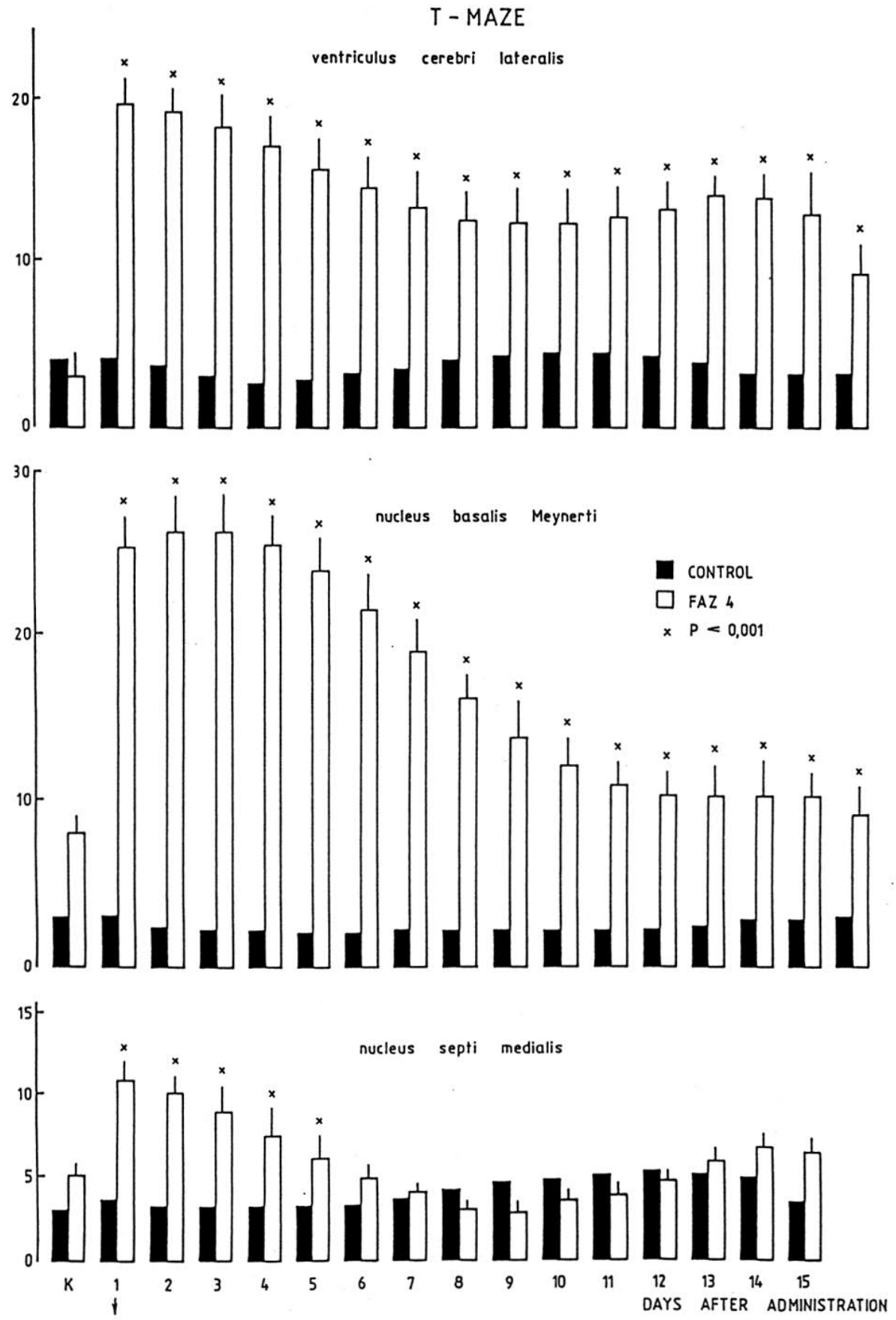

Fig. 3 Effect of FAZ-4 in a dose of 0.3 mg i.c. on spatial orientation of animals tested in the T-maze

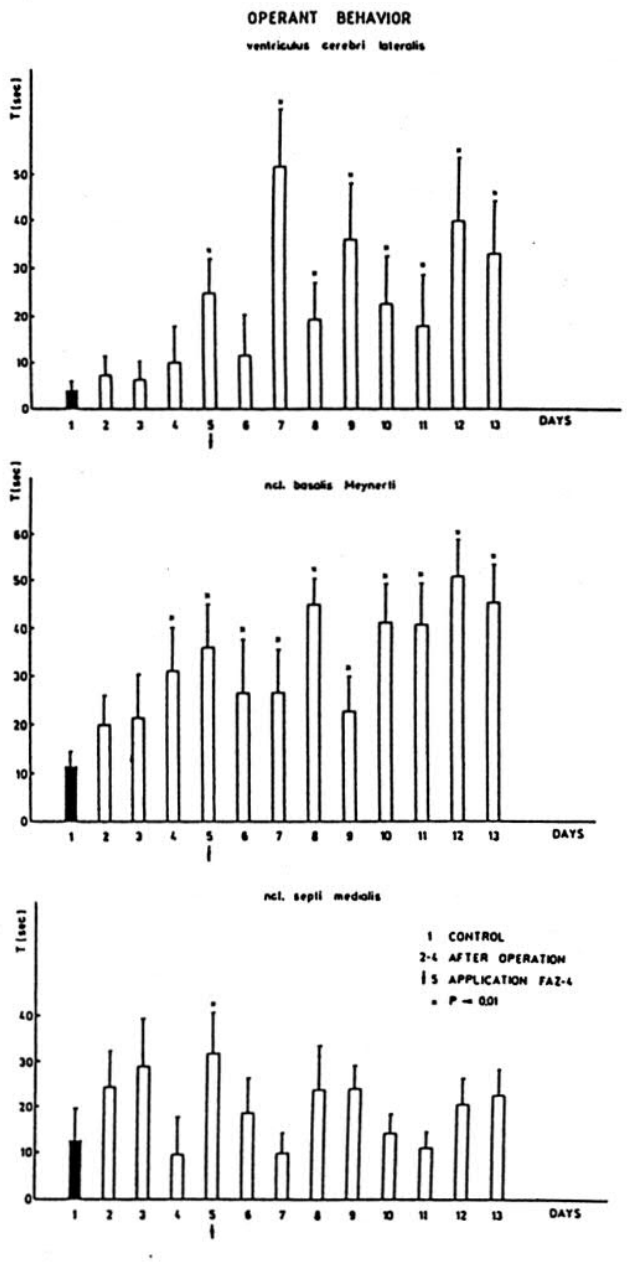

Fig. 4 Effects of FAZ-4 in a dose of 0.3 mg i.c. on operant behaviour on animals in the Skinner's box

15-21 days after drug administration. A lower dose of 0.3 mg did not induce negative changes in the reactions under study in rats. After administration of the drug, the first days of the experiment both groups of animals showed shorter latency time of measured reactions in comparison with controls.

The rate of recall from memory in the test of orientation in space was evaluated also in the animals whom FAZ-4 (0.30 mg) was administered i.c. This dose produced significant changes in the behaviour of animals. Negative effects of FAZ-4 became manifested particularly in the time determining the rate of orientation of animals in space as well as in the number of errors measured by the number of enterings into the individual branches of the labyrinth. The rate of orientation of animals and their wandering were in correlation. The effect of FAZ-4 depended on the selection of the structure into which it was administered. Its negative action manifested by decreased ability of animals to react to stimuli was observed after administration into the Meynert's nucleus and the lateral cerebral ventricle. Minor changes in tested reactions were observed in its administration into the medial septum (Fig. 3) in the first days of the experiment. Operant behaviour of animals was influenced by FAZ-4 after its administration into all three structures. Animals were tested under control conditions prior to the operative introduction of the guides of the cannulae, then after the operation, and finally after the administration of the durg (Fig. 4). The time of accession of the animals to the food-house and bringing it into operation was measured in 13 sessions. As follows from Fig. 4, the performance of the operation did not influence the investigated behaviour of animals. It further follows from the result of the experiment that FAZ-4 negatively influenced recall from memory in those animals which received it into the ncl. basalis Meynerti and the lateral cerebral ventricle. After its administration into the medial septum only slight changes in the recall from memory were observed on the day of its administration.

The test of spatial orientation of animals in the T-maze examined the central effects of DSP-4 administered in a dose of 1 mg in 3 μl of NaCl, 0.9 %, into three cerebral structures. Different effects of the drug were observed in dependence on the localization of administration. Memory retention was tested 30 and 90 min after its administration and then daily for a period of 9 days. Negative effects on memory after DSP-4 became manifested most markedly in the first time period after its administration in the animals to which it was administered into the lateral cerebral ventricle and the Meynert's nucleus. Its effects via the septum appeared later, worsening the ability of recalling from memory. In all animals tested, the disorders of memory were normalized in a few days (Fig. 5).

Effects of DSP-4 were further examined with regard to the animals' ability of escaping a painful stimulus. The first observations carried out 30 and 90 min after drug administration reveal a significant damage in the region of the Meynert's nucleus which is manifested by deteriorated recall from memory. Similar effects manifested by bad recall from memory were produced by DSP-4 in animals whom this drug was administered into the lateral cerebral ventricle and they were observed beginning the second day after administration. A return

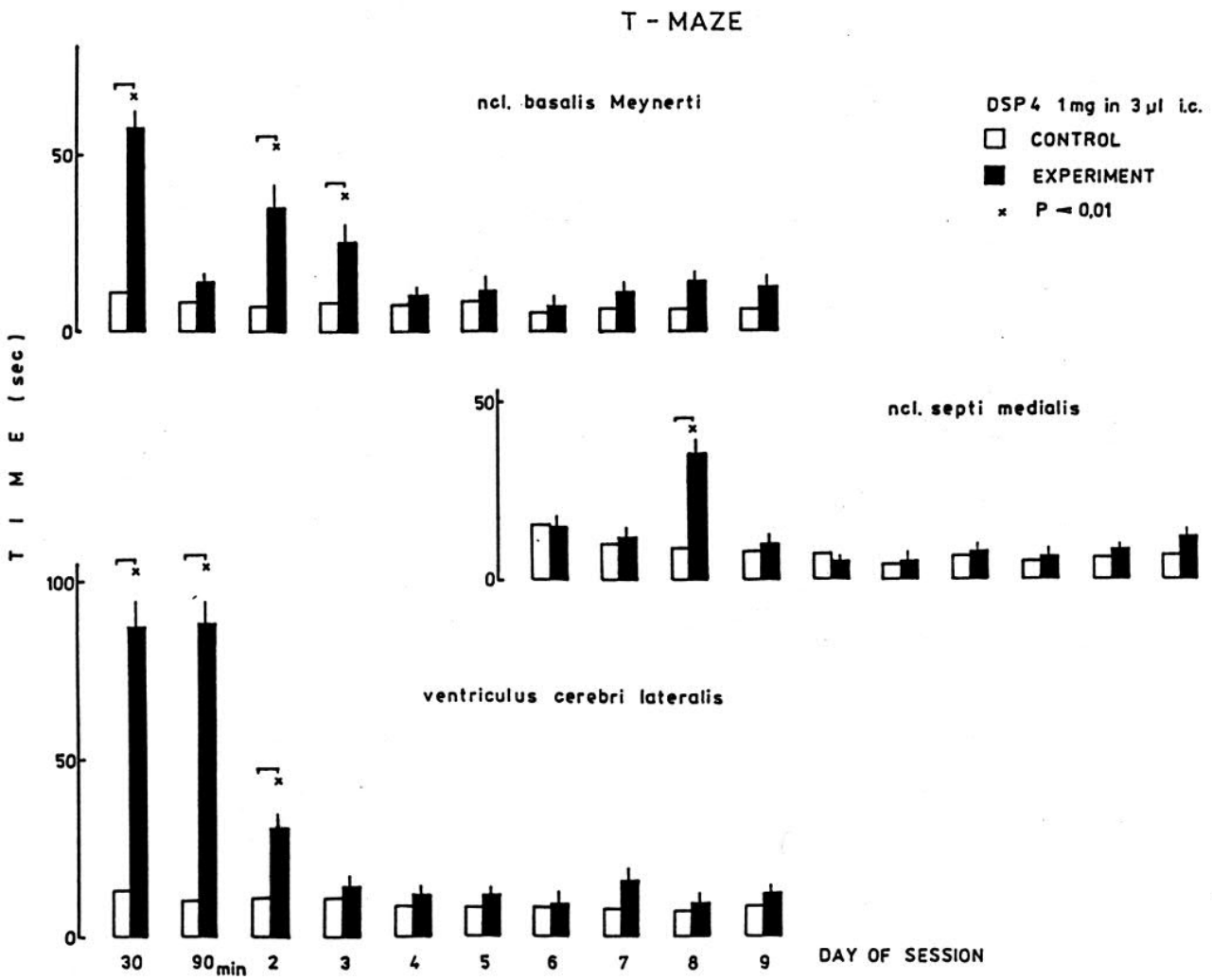

Fig. 5 Effects of DSP-4 in a dose 1.0 mg i.c. on spatial orientation of animals tested in the T-maze

to normal values was observed one week after drug administration. The animals whom DSP-4 was administered into the medial septum did not show disorders of memory. The results of the experiment are presented in Fig. 6.

The dashed line marks the time limit when the animal can still run away into the safe part of the box to escape the electric shock.

Discussion

In their previous papers, the present authors demonstrated a capability of mescaline and FAZ-4 to impair spatial orientation and movement of experimental animals in a free field after systemic administration (7, 8). Attempting to obtain further information about possible sites of intervention of neurotoxins in the brain, a local mode of administration into selected cerebral structures was selected in the present study. The septum and the ncl. basalis Meynerti (NBM) represent the beginning of two principal cholinergic systems of the brain (10, 20). The role which the septum plays for the hippocampus is analogical to that of the NBM for the neocortex. At present the key role of the ncl. basalis Maynerti in the etiopathogenesis of dementia of Alzheimer's type is recognized, which holds true particularly for early stages of this disease (12, 19). In addition, the results of scintigraphic analysis of accumulation of the derivative ^{3}H-FAZ-1 after intracerebroventricular administration demonstrated a marked increase in its accumulation just in the septum (13). Deterioration of spatial orientation in the cases of both FAZ-4 and DSP-4 was, however, more marked after administration of these neurotoxins into the lateral cerebral ventricle and into the NBM. Operant conditioning was impaired in the same manner. On the other hand, the region of the septum was less sensitive from the viewpoint of

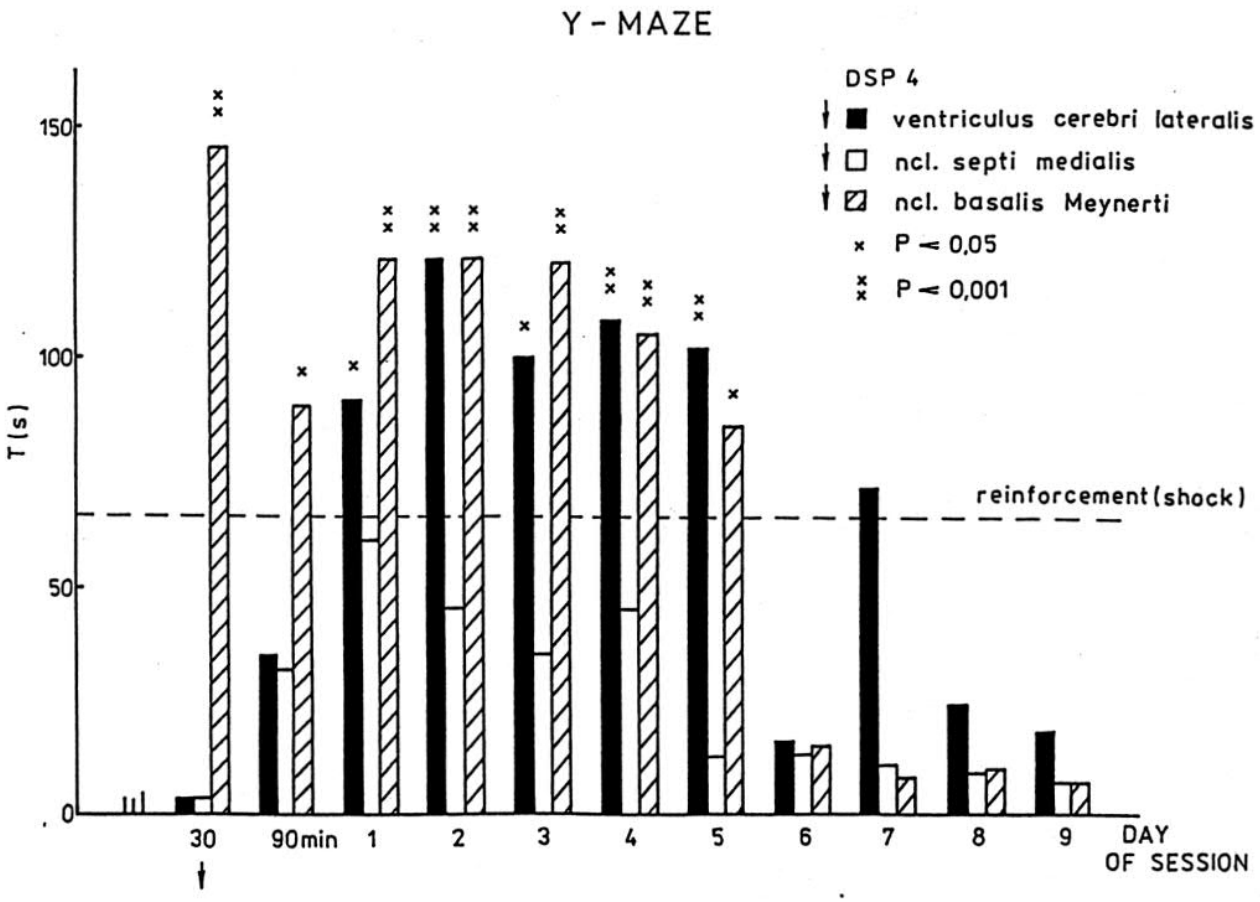

Fig. 6 Effects of DSP-4 in a dose of 1.0 mg on the defensively reflexive behaviour of animals in the Y-labyrinth

inducement of disorders of the reactions under study. An explanation could be provided by our previous histoneuropathological findings after intracerebral administration of FAZ-4 (5). Destructive changes in the cerebral tissue outlasted for several weeks. The necrotic foci at the boundary of the lower and medial part of ncl. dorsalis corporis geniculati lateralis, typical for neurotoxin administration into the lateral ventricle and the NBM, could give evidence for an impairment of the final neuron of the optic tract. After neurotoxin administration into the septal region these changes were not found. The affected animals thus probably suffered from partial cortical blindness, which could be negatively reflected also in their behavioral performance. Mescaline showed the most marked suppressive effect on spatial behaviour of experimental animals after administration into the medial septum. On the other hand, administration into the lateral cerebral ventricle was least effective. These changes make us think of possible participation of monoaminergic neural transfer in relation to mescaline. A generally assumed interaction between the cholinergic and noradrenergic systems in the mechanisms of memory was recently summarized by, e.g. Altman (1). In the tests of spatial orientation, a two-stage effect of mescaline was further observed, which consisted of initial prolongation of measured latency time, followed by a certain modification, which was followed by another period of prolonged latency. This multi-stage effect was observed in the case of active evasive reactions by Davis and Haton (2). An explanation of this effect could be gradual intervention of mescaline into more than only one neuronal mechanism. The first one would react „immediately", i.e. in hours, maximally days after single-dose administration of the drug. Other reactions are „delayed" after latency of more than one week. The basis of the first mechanism could be a direct intervention predominantly at the receptor level, whereas the „delayed" mechanism probably reflects the proper destructive changes of the whole neuronal populations induced by the neurotoxic effect.

Also the results of experiments with DSP-4 make us think of participation of the central noradrenergic mechanism. A number of data from the literature confirm participation of this system in both spatial orientation and operant conditioning (11, 16, 17, 18, 22). Depletion of NA after DSP-4 administration generally results in a decrease of searching activity, a decreased reaction to novelty, and increased latency of drawing nearer (3). On the basis of the

present author's data it is obvious that some qualities of behaviour may be impaired after damaging both the cholinergic and the monoaminergic central structures.

References

1. ALTMAN, HL. - STONE, WS. - ÖGREN, SO: Evidence for a possible functional interaction between serotonergic and cholinergic mechanisms in memory retrieval. Behav., Neural., Biol., 1987, vol. 48, p. 49-62.
2. DAVIS, WM. - HATOUM, HL.: Comparison of stimulants and hallucinogens on shuttle avoidance. Gen. Pharmac., 1987, vol. 18, p. 123-128.
3. DELINI-STULA, A., et al.: Novelty-oriented behavior in the rat after selective damage of locus coeruleus projection by DSP-4, a new noradrenergic neurotoxin. Pharmacol. Biochem. Behav., 1984, vol. 20, p. 613-618.
4. GALLO, A., et al.: Topographic analysis of the rat's bar behaviour in the Skinner box. Behav. Process, 1995, vol. 33, p. 319-328.
5. HERINK, J. - KOUPILOVÁ, M. - KRS, O. - BAJGAR, J. - PATOČKA, J.: Modelling of some neuropathological states of the central nervous system by aziridine derivatives. Čs. Fyziol., 1992, vol. 41, p. 7-10.
6. HERINK, J.: Convulsive properties of N-(3,5-dimethoxy-4--propoxyphenyl-ethyl) aziridine and their influencing by diazepam and triazolam. Sbor. věd. Prací LF UK Hradec Králové, 1995, vol. 38, p. 85-88.
7. KOUPILOVÁ, M. - HERINK, J.: Effects of mescaline and its derivative n-(3,4,5-trimethoxyphenylethyl)-aziridine on the spatial orientation of rats in a t-maze. Physiol. bohemoslov., 1989, vol. 38, p. 497-502.
8. KOUPILOVÁ, M. - HERINK, J. - BAJGAR, J.: Effects of aziridine derivative N(3,5-Dimethoxy-4-propoxyphenylethyl)--aziridine on learning and memory in laboratory rats. Homeostasis, 1993, vol. 34, p. 117-119.
9. MARHOLD, J.: Přehled průmyslové toxikologie. Praha, Avicenum, 1986. 815 s. Organické látky. Sv. 2.
10. MORAN, PM., et al.: Reversal of learning and memory impairment following lesion of the nucleus basalis magnocellularis (NBM) by concurrent noradrenergic depletion using DSP-4 in the rat. Brain Res., 1992, vol. 595, p. 327-333.
11. MORLEY, MJ., et al.: DSP-4 and Herrnstein's equation: further evidence for a role of noradrenaline in the maintenance of operant behaviour by positive reinforcement. Psychopharmacology, 1988, vol. 96, p. 551-556.
12. OLTON, DS. - WENK, GL.: Dementia: Animal models of the cognitive impairments produced by degeneration of the basal forebrain cholinergic system. In Meltzer, HY. (Ed.), Psychopharmacology, 3rd generation of progress. New York, Raven Press, 1987, p. 941-953.
13. PAŘÍZEK, J. - SLÍŽOVÁ, D.: Histochemická verifikace. Psychopharmacol. Meeting, Jeseník Spa, January, 1988.
14. POPE, CN. - HO, BT. - WRIGHT, AA.: Neurochemical and behavioral effects of N-ethyl-acetylcholine aziridinium chloride in mice. Pharmacol. Biochem. Behav., 1987, vol. 26, p. 365-371.
15. RAZDAN, RK.: U.S.P. 3, 637, 662. Patented Jan. 25. 1972.
16. SANTUCCI, AC. - HAROUTUNIAN, V. - DAVIS, KL.: Pharmacological alleviation of combined cholinergic/noradrenergic lesion-induced memory deficits in rats. Clinic. Neuropharm., 1991, vol. 14, p. 1-8.
17. SIRVIÖ, J., et al.: The effects of noradrenergic neurotoxin, DSP-4, on the performance of young and aged rats in spatial navigation task. Brain Research, 1991, vol. 563, p. 297-302.
18. TAKASUNA, M. - IWASAKI, T.: Active and passive avoidance learning in rats neonatally treated with intraventricular 6--hydroxydopamine. Behav. Brain Res., 1996, vol. 74, p. 119-126.
19. TOLEDANO-GASCA, A.: Hypothesis concerning the etiology of Alzheimer's disease. Pharmacopsychiat., 1988, vol. 21, p. 17-25.
20. TUČEK, S. - DOLEŽAL, V. - NEDOMA, J.: Cholinergic mechanisms in the brain. Activ. nerv. sup. (Praha), 1986, vol. 28, p. 42-43.
21. WENGER, GR.: Operant behavior as a technique for toxicity testing neurotoxicol. Teratol., 1990, vol. 12, p. 515-521.
22. ZAGRODZKA, J. - WIECZOREK, M. - ROMANIUK, A.: Social interactions in rats: behavioral and neurochemical alterations in dsp-4-treated rats. Pharmacol. Biochem. Behav., 1994, vol. 49, p. 541-548.

Correspondence: RNDr. Marie Koupilová, CSc.
Vojenská lékařská akademie J. E. Purkyně
Třebešská 1575
500 01 Hradec Králové

Received: 19. 10. 1998