Early Postnatal Effects of Noopept and Piracetam on Declarative and Procedural Memory of Adult Male and Female Rats

S. S. Trofimov, T. A. Voronina, and L. S. Guzevatykh

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We studied the effect of a new nootropic dipeptide Noopept and reference nootropic preparation piracetam injected subcutaneously on days 8-20 of life on learning of alternative feeding response in a 6-arm-maze in male and female rats. Early postnatal administration of Noopept disturbed the dynamics of learning by parameters of declarative and procedural memory. Piracetam impaired learning by parameters of procedural, but not declarative memory (only in males). Both preparations decreased the ratio of successfully learned males (but not females). The observed effects were not associated with changes in locomotor activity.

Key Words: *nootropic agents; peptides; piracetam; Noopept; ontogeny*

Nootropic drugs are now often prescribed to young children with perinatal encephalopathy. However, many authorities think, that this approach is not justified, because hyperdiagnosis often leads to overtreatment [3] and sometimes conventionally healthy children not requiring pharmacotherapy have to take these drugs. Delayed consequences of nootropic treatment during the early postnatal ontogeny remain unknown. Clinical trials of the effects of a new nootropic peptide Noopept (ethyl ester of N-acetyl-L-prolyl glycine) in adult people are now in progress [5]. The development of possible delayed changes in mnestic functions was studied in adult rats receiving Noopept or reference preparation piracetam during the early postnatal ontogeny.

MATERIALS AND METHODS

Experiments were performed on outbred male and female albino rats. The next day after finding newborn rat pups in the cage was considered as day 1 of life. Noopept (0.1 mg/kg) and piracetam (200 mg/kg, 1 ml/100 g) were injected subcutaneously on days 8-20 of life (13 injections). Control rat pups received an equivalent volume of 0.9% NaCl.

Training in the 6-arm-maze (Fig. 1) was performed with adult rats aging 2 months. The animals were deprived of food before training. Each animal was placed in the start chamber (bread crump balls were scattered over the floor of the maze). The rat explored the maze for 15 min and then was removed. On the next day, the reinforcement was put on a shelf in arm A. The rat was placed in the start chamber. When the rat found and ate the food or failed to find the rein-

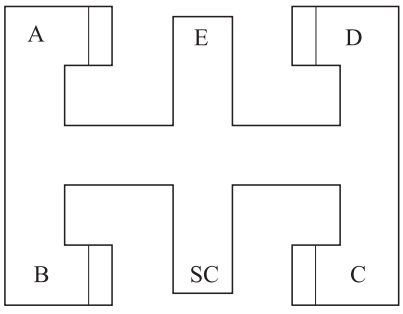

Fig. 1. Scheme of 6-arm-maze. SC, start chamber; A-E, arms of the maze.

Laboratory of Psychopharmacology, V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** ap_natalia@mail.ru. S. S. Trofimov

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forcement for 15 min, it was removed from the maze and placed again in the start chamber. In this case, the reinforcement was placed in the contralateral part of a chamber (arm C). Location of the reinforcement in consecutive trials was alternated (arm A, arm C, *etc*.). The same procedure was repeated on the next day. The reinforcement was subsequently put in arms A, C, A, *etc*. In experiments with another rat, the reinforcement was put in arms B and D. Each rat was placed in the maze 6 times a day. Learning was performed for 8 days. When the rat was placed in the maze on the next experimental day, it should remember spatial location of arms and the way to reinforcement (declarative memory). Moreover, the rat should remember alternation of arms (working and procedural memory). Two parameters were evaluated at each stage of learning (1st, 2nd, and 6th runs): the time reaching reinforcement after leaving the start chamber and number of entries into empty arms (errors). The number of rats attaining the learning criterion (finding reinforcement in each trial, *i.e.* 6 times a day, and without errors, *i.e*., entries into empty arms) was also evaluated.

Spontaneous locomotor activity was studied after 8-day learning. The rats were deprived of food for 1 day and placed in an Opto-Varimex device (Columbus Instruments). Horizontal activity was recorded at 1-min intervals.

The results were analyzed by one-way dispersion analysis (ANOVA). Pairwise comparison of statistically significant data involved Newman—Keuls test. The data were also processed using exact Fischer test [7].

RESULTS

Evaluation of the dynamics of learning of feeding response in the 6-arm-maze revealed no differences between control males and females (Fig. 2, Table 1).

Evaluation of the dynamics of learning by the parameters of declarative memory in animals receiving piracetam or Noopept in the early postnatal ontogeny revealed no significant changes in the number of errors (Fig. 2, *a*) and by the time of attaining the reinforcement (Table 1). Analysis of the effect of the test drugs on procedural memory showed that piracetam

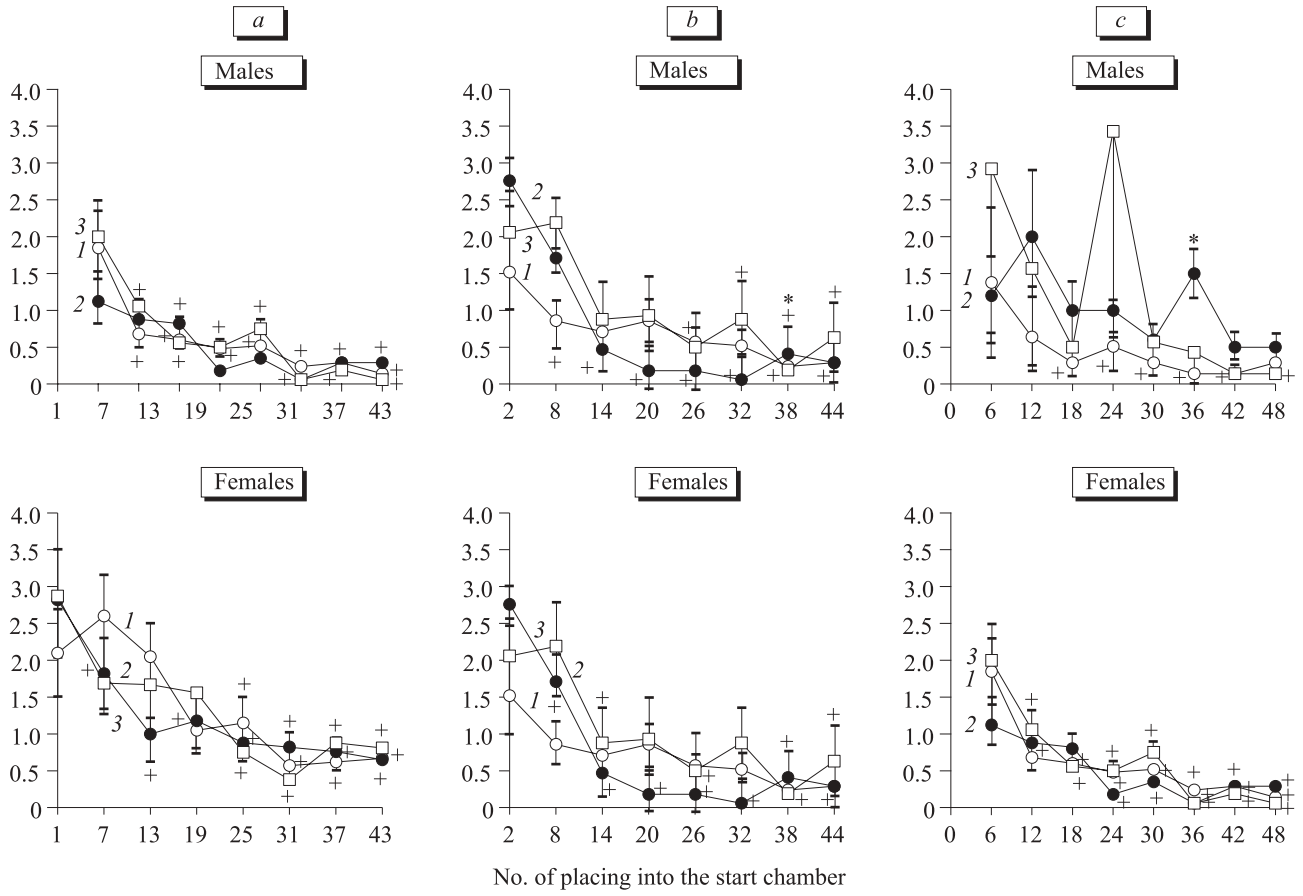

Fig. 2. Dynamics of learning in male and female rats. Number of errors (entries into empty arms): 1st (*a*), 2nd (*b*) and 6th runs in each day over 8-day learning (*c*). Control animals receiving 0.9% NaCl on days 8-20 of life (*1*); postnatal treatment with piracetam (*2*) or Noopept (*3*). **p*<0.05 compared to control animals of the same sex in the same trial; +*p*<0.05 compared to the 1st, 2nd, or 6th trial with the same group.

TABLE 1. Time of Running to Reinforcement for Adult Rats during Learning in 6-arm-Maze (sec)

Group		Trial number (1st of 6 trials in the corresponding day)							
		1	7	13	19	25	31	37	43
0.9% NaCl	males	140.1±25.2	72.0±16.0$^+$	30.9±8.8$^+$	11.4±3.3$^+$	7.1±1.5$^+$	10.9±4.5$^+$	6.4±2.0$^+$	5.9±1.5$^+$
	females	100.6±20.1	109.7±21.2	48.9±14.7$^+$	50.7±19.3$^+$	37.4±14.5$^+$	23.4±13.6$^+$	7.7±1.7$^+$	7.2±1.9$^+$
Piracetam	males	115.5±32.3	102.4±25.1	81.1±36.2	77.0±37.0	39.6±26.4	35.9±26.6	18.2±5.6$^+$	14.4±4.8$^+$
	females	127.2±23.6	60.3±15.3$^+$	22.7±5.9$^+$	19.5±5.7$^+$	17.9±4.9$^+$	10.7±2.5$^+$	9.1±3.1$^+$	5.2±1.1$^+$
Noopept	males	139.1±28.2	71.6±24.4$^+$	22.8±6.0$^+$	43.8±18.1$^+$	17.9±7.7$^+$	10.4±2.0$^+$	9.3±3.4$^+$	8.5±3.7$^+$
	females	132.1±27.9	64.9±17.7$^+$	42.0±17.9$^+$	22.5±5.8$^+$	13.8±3.8$^+$	6.6±2.0$^+$	9.8±2.1$^+$	7.8±1.6$^+$
		Trial number (2nd of 6 trials in the corresponding day)							
		2	8	14	20	26	32	38	44
0.9% NaCl	males	92.9±24.6	33.3±10.8$^+$	7.2±2.3$^+$	5.9±2.9$^+$	9.6±5.3$^+$	1.7±0.2$^+$	24.1±20.3$^+$	2.4±0.8$^+$
	females	77.6±21.9	45.9±15.0	27.5±10.3$^+$	24.0±14.0$^+$	21.4±11.8$^+$	10.9±4.7$^+$	3.8±0.8$^+$	5.5±1.7$^+$
Piracetam	males	95.1±30.8	84.2±37.5	65.5±38.9	96.1±44.4*	37.9±29.3	13.1±6.9*	13.0±4.6	3.3±0.9
	females	150.5±25.9	53.3±17.6$^+$	7.5±3.1$^+$	3.8±1.6$^+$	3.5±1.4$^+$	2.1±0.3$^+$	4.3±1.6$^+$	2.9±0.8$^+$
Noopept	males	56.3±19.1	43.3±15.2	4.9±1.5$^+$	30.0±19.1	2.1±0.3$^+$	2.1±0.2$^+$	2.6±0.6$^+$	2.7±0.6$^+$
	females	79.3±18.4	46.3±14.8$^+$	11.7±3.8$^+$	30.6±18.5$^+$	5.2±1.6$^+$	9.3±4.2$^+$	2.3±0.5$^+$	4.0±1.4$^+$
		Trial number (6th of 6 trials in the corresponding day)							
		6	12	18	24	30	36	42	48
0.9% NaCl	males	68.0±25.3	8.8±3.5$^+$	5.1±2.0$^+$	3.1±0.7$^+$	4.3±1.3$^+$	2.6±1.1$^+$	4.9±3.0$^+$	3.6±1.7$^+$
	females	108.4±23.5	44.6±19.7	22.7±14.3$^+$	4.6±1.2$^+$	5.2±1.7$^+$	3.3±0.9$^+$	2.7±0.5$^+$	27.1±4.3$^+$
Piracetam	males	113.2±38.8	73.9±37.1*	40.3±28.9	44.2±29.8	5.5±2.1$^+$	8.2±3.2$^+$	6.2±2.2$^+$	3.1±0.7$^+$
	females	55.7±19.4	22.8±12.7$^+$	8.3±2.9$^+$	3.0±1.0$^+$	1.9±0.5$^+$	1.8±0.2$^+$	4.1±1.6$^+$	3.5±1.1$^+$
Noopept	males	88.4±26.1	26.9±9.1$^+$	4.6±1.3$^+$	19.1±12.0$^+$	4.9±1.8$^+$	2.9±0.8$^+$	2.0±0.5$^+$	2.2±0.6$^+$
	females	70.4±21.6	29.8±17.2$^+$	15.0±10.0$^+$	5.4±1.5$^+$	6.0±1.3$^+$	2.9±0.6$^+$	2.7±0.7$^+$	2.8±1.1$^+$

Note. Here and in Table 2: *$p<0.05$ compared to 0.9% NaCl; $^+p<0.05$ compared to the 1st, 2nd, or 6th trial in the same group.

TABLE 2. Dynamics of Learning in Male and Female Rats (Ratio of Animals Attaining Learning Criterion, %)

Group		Day of learning							
		1	2	3	4	5	6	7	8
Males	0.9% NaCl	0	0	14.3	21.4	50^{+}	64.3^{+}	85.7^{+}	92.9^{+}
	Piracetam	0	0	10	30	30	30	30*	30*
	Noopept	0	0	14.3	14.3	21.4	38.6	35.7*	35.7*
Females	0.9% NaCl	0	0	4.8	23.8^{+}	23.8^{+}	38.1^{+}	42.9^{+}	47.6^{+}
	Piracetam	0	0	11.8	17.6	47.1^{+}	47.1^{+}	47.1^{+}	58.8^{+}
	Noopept	0	0	6.3	12.5	25	43.8^{+}	50^{+}	56.3^{+}

impaired the performance and increased the number of errors (36th and 38th trials); noopept only slightly increased the number of errors (24th trial, Fig. 2, *b*, *c*). Both preparations had no effect on learning in females by the parameters of procedural memory.

The learning criterion serves as the most reliable end-point. We showed that 93% control males and 48% control females reached the learning criterion, but this difference was statistically insignificant.

Comparison by the learning criterion did not revealed no differences between control females and females receiving Noopept or piracetam during the early postnatal ontogeny (Table 2). However, the number of males receiving Noopept or piracetam and reaching the learning criterion was much lower compared to the control (Table 2). As distinct from control rats, the percent of experimental animals reaching the learning criterion did not increase even in the last day of training (day 8).

The differences in maze behavior of rats can be associated with different locomotor activity. However, behavioral studies on an Opto-Varimex device revealed no significant changes in horizontal activity between these groups (Table 3).

Treatment with a substance during the early ontogeny (formation and activation of individual functional systems) modulates various developmental processes. Activation and inhibition of systems modulate their function in the delayed period, simulate natural changes in afferentation, and reproduce the effect of pathogenic or toxic factors [9]. These mechanisms probably underlie the influence of Noopept and piracetam. Published data show that Noopept modulates activity of the cholinergic system [6]. Metabolism of this substance yields various compounds, two of which serve as endogenous metabolites in rats (phenylacetic acid and cyclo-Pro-Gly) [1,8]. Piracetam modulates all neurotransmitter systems, but is excreted in native state [2].

Difficulties in maze learning after treatment with study preparations were observed in males, but not in females. This attests to the involvement of hormonal mechanisms into the development of mnestic defects, which agrees with the hypothesis that individual variations in the nootropic effect of piracetam can be related to individual hormonal differences [10].

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TABLE 3. Locomotor Activity of Rats Receiving 0.9% NaCl, Piracetam, or Noopept (M±m)

Time of recording, min	0.9% NaCl		Piracetam		Noopept	
	males	females	males	females	males	females
1	562.3±66.6	664.8±33.7	465.0±56.6	624.3±73.2	438.3±60.4	596.3±68.2
2	384.0±22.6	362.1±43.7	275.6±45.2	339.8±42.0	309.2±45.3	362.1±43.7
3	357.0±34.5	253.4±33.3	283.6±29.9	330.9±35.6	308.4±53.0	250.8±49.6
4	222.6±27.7	296.5±35.1	241.7±35.1	263.2±29.4	314.9±26.9	223.4±32.7
5	244.1±45.1	262.8±43.3	239.5±62.7	269.3±41.5	251.2±45.0	184.8±26.1
6	181.9±31.4	219.3±39.2	119.3±37.0	202.2±41.7	225.1±37.5	215.8±34.8
7	156.3±41.8	203.6±39.8	128.3±40.6	109.2±29.2	180.3±48.2	155.4±35.9
8	132.0±47.2	152.7±33.1	178.2±42.1	140.8±31.2	279.2±40.8	172.0±34.4
9	128.4±41.6	97.7±27.8	115.8±25.9	116.5±26.4	199.4±46.5	167.7±36.6
10	151.4±35.9	95.1±26.2	100.2±35.0	73.0±24.5	145.1±69.0	155.8±31.9
10-min activity	2573.6±223.1	2546.0±204.2	2155.0±248.5	2469.3±154.9	2711.4±240.1	2484.1±195.5

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