Behavioral Effects of Original Tetrapeptide, an Analog of N-Terminal Nociceptin Fragment

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The study examined the effect of an analog to N-terminal nociceptin fragment AcOH×Phe-Gly-Gly-Phe-NH_2 on the behavior of albino rats. This tetrapeptide (5 μg/kg intraperitoneally) significantly enhanced motor and exploratory activity in mature rats and in 42-day pups and produced opposite effects in 21-day rat pups, which attests to the complex dynamics of maturation of nervous structures involved in the realization of nociceptin action.

Key Words: *N-terminal fragment of nociceptin; motor activity; exploratory behavior; anxiety*

Nociceptin (orphanin FQ), a 17-amino acid neuropeptide, is similar to endorphins by chemical structure. Structurally, it differs from the classical opioid peptides by N-terminal phenylalanine instead of tyrosine. Nociceptin is endogenous ligand of ORL_1 receptor, which is roughly 50% homological to opioid μ-, δ-, and κ-receptors. According to current views, the major physiological role of nociceptin is modulation of nociception (pain reception): supraspinal injection of this peptide produces hyperalgesia, while spinal injection results in analgesia [7,13]. In rodents, central injection of nociceptin affects various behavioral modalities such as food consumption, motor activity, anxiety, and learning ability [7,10,16]. The direction of peptide action can change depending on the dose, mode of administration, species, strain, and sex of the experimental animals [6-8,13]. Specifically, intraperitoneal nociceptin exerts both anxiolytic and anxiogenic effects [9,12].

The behavioral effects of systemically applied nociceptin or its fragments are virtually unknown. It is also true for N-terminal tetrapeptide Phe-Gly-Gly-Phe that is responsible for binding to ORL_1 receptor [11]. The studies of these behavioral effects are of theoretical and practical importance (in view of possible clinical use of nociceptin fragments).

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MATERIALS AND METHODS

The experiments were carried out on mature randombred rats (n=59; 31 males and 28 females, age >90 days) and rat pups (n=55; 27 males and 28 females).

The analog of N-terminal nociceptin fragment AcOH×Phe-Gly-Gly-Phe-NH_2 was synthesized in Chemistry Faculty of St. Petersburg State University. The rats of experimental group received tetrapeptide at the dose of 5 mg/kg chosen according to our previous studies with opioid peptides [2]. The drug was dissolved in distilled water containing 3.33% alcohol (1 ml/kg for mature rats and 2 ml/kg for pups). The control rats received an equivalent volume of the solvent. The number of males and females in control and experimental groups was equal. The injections were made intraperitoneally 20 min before behavioral testing. The reactions were examined in an open field made of a round plastic arena 100 cm in diameter encircled with a high wall (OpenScience). The marking lines (two circles and the sector lines) divided the arena into 19 segments of equal area. There were 13 holes at the arena center and on the sector lines. Red (15 W) and bright (60 W) lamps were positioned 80 cm above the arena center. In the tests with mature

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rats, illumination was changed as follows: bright light (3 min), red light (1 min), and bright light (1 min) [4]. Another protocol was chosen in the experiments with rat pups: bright light (3 min), red light (3 min), and bright light (3 min). The pups were tested at the age of 21 and 42 days (the drug was injected in both cases). Every minute during testing, the following parameters were measured: *1*) horizontal motor activity (number of crossed arena segments); *2*) vertical activity (number of rearing events); *3*) numbers of crossing the external and internal circles (departures from arena wall and visits to arena center); *4*) number of grooming episodes; *5*) number of explored holes; and *6*) number of defecations. The data were analyzed statistically using ANOVA software. Significance was assessed by Mann–Whitney, χ^2, and Student's *t* tests.

RESULTS

Effect of tetrapeptide on behavior of mature rats. The experiments revealed activation effect of N-terminal fragment of nociceptin on the motor and explorative behavior of mature rats. In experimental rats, the absolute values of behavioral parameters recorded in the open field with alternating illumination significantly surpassed the control.

The total running length of the rats recorded during 5 minutes in control and experimental groups were respectively, 59.8±5.6 and 70.8±5.7 segments (*m*±SEM). Moreover, the runs greater than 80 segments were demonstrated by 3 of 28 control rats and 11 of 31 experimental animals ($p<0.05$ by χ^2 test). In addition, the running length measured during the fifth minute of the test was significantly larger in the experimental rats compared to controls (Fig. 1, *a*). The total numbers of rearing events were 17.3±1.8 and 21.2±2.1 in the control and experimental groups, respectively. The numbers or rearing postures surpassed 30 in 1 control and 7 experimental rats ($p<0.05$ by χ^2 test).

The total numbers of visits to arena center were 2.8±0.2 and 1.9±0.1 in experimental and control rats, respectively ($p<0.05$ by Student's test), the difference being most pronounced on the test minute 4 (Fig, 1, *b*). During the entire observation period, the experimental rats more frequently walked away from the arena wall, and this trend became significant on test minute 5 (Fig. 1, *c*). However, we did not observe any intergroup differences in the numbers of grooming and defecation events.

Increase in the running length, the number of rearing events, and in the number of departures from the arena wall in the experimental group attest to activation of motor and exploratory activity by the examined tetrapeptide. It is noteworthy that the greatest intergroup differences were documented on the test minutes 4-5, *i.e.* after illumination had been changed from bright to red thereby moderating the environmental stressogenicity and potentiating the orientation and exploratory behavior.

The species-specific exploratory activity assessed in the rodents by the number of explored holes was also higher in the experimental group (3.0±0.5 *vs*. 2.2±0.2 in the control). None of control rats explored more than 4 holes, while in the experimental group 9 rats demonstrating such elevated exploratory activity ($p<0.01$, χ^2-test). The greatest intergroup difference in the number of explored holes was revealed on test minute 5 (Fig. 1, *d*).

The data analysis by ANOVA with consideration of "sex" and "group" factors as well as the results of repeated measurements made every minute, showe that the tetrapeptide increased the number of rearing events predominantly in males, while experimental females demonstrated greater exploratory activity (hole exploration). In other words, the sex difference was significant for both behavioral parameters ($p<0.05$). Examination of other behavioral parameters (including the tests with the pups) revealed no significant gender differences.

Age-dependent behavioral effect of tetrapeptide on the pups. One of the aims of this study was assessment of age-related effects of the examined peptide on rat behavior. To this end, the experiments were carried out not only on mature rats, but also on 21- and 42-day-old pups. The testing periods were selected based on our previous studies of the behavioral activity of the opioid peptides [1].

At the age of 3 weeks, there were no significant difference in horizontal and vertical motor activities between the control and experimental groups. However, the experimental pups were characterized by significantly lower number of departures from the arena wall (the total number of 1.8±0.3 in comparison with the control value of 2.9±0.4; $p<0.05$ by Mann–Whitney test). Under red light (test minute 4-6), this phenomenon was most pronounced (Fig. 2, *a*). Moreover, under this illumination the experimental pups explored less number of the holes (Fig. 2, *b*).

Thus, exploratory activity in rats treated with an analog to N-terminal nociceptin fragment at the age of 21 days was reduced. Since most studies consider the number of departures from arena wall as an indicator of anxiety, our data attest to certain anxiogenic effect of the tetrapeptide on rat pups during the examined ontogenetic period.

The 6-week control and experimental rats demonstrated no significant difference in the numbers of runs and rearing events. There were no signs of inhibited explorative activity and increased anxiety in the rat pups, although both effects were revealed during the first test made at the age of 21 days. On the contrary,

in 42-day pups the behavioral effect of tetrapeptide became similar to that observed in the mature rats, which attests to biphasic dependence of this effect on age.

The experimental pups visited the arena center more often than the controls (2.1±0.4 and 1.4±0.3, respectively). At this, 4 of 27 pups treated with tetrapeptide visited the arena center at least 5 times. In the control group (n=28), there were no such pups at all (p<0.05 according to χ^2 test). The most pronounced differences in the number of visits to arena center were documented under red light (Fig. 2, *c*). On test minute 2, the experimental pups demonstrated significantly greater number of the examined holes: 1.3±0.3 in comparison with the control value 0.6±0.2 (p<0.05 according to Student's test, Fig. 2, *d*). However, although the tetrapeptide elevated explorative activity in the 42-day pups, this effect was significantly less pronounced than that in mature rats.

It is an established fact that during the first two postnatal weeks, expression of RNA of the nociceptin precursor protein in rat pups brain equals to that in the mature rats; the same is true on mRNA expression of ORL_1 receptors [14]. However, our data attest to inversion of the effect of the examined tetrapeptide in the time interval between 21 and 42 days of rat life. Thus, the "mature" level of concentration of a neurotransmitter and its receptors cannot be considered as the sign of completion of brain maturation, where the corresponding synapses may not be yet formed in the final form. Interestingly, in the control group a strict correlation was found between the numbers of departures from the arena wall recorded under red light in 21- and 42-day pups (r=0.59; p<0.01), whereas virtually no correlation between these parameters was observed in experimental pups (r=0.09). Thus, 3- and 6-week rat pups differently reacted to injection of the tetrapeptide, which attests to continuation of maturation of ORL_1-system during this postnatal period.

Our findings agree with published reports [12] that intraventricular injection of 0.1-3.0 nM nociceptin decreases anxiety of Wistar rat males in the tests under bright illumination. Moreover, the revealed behavioral

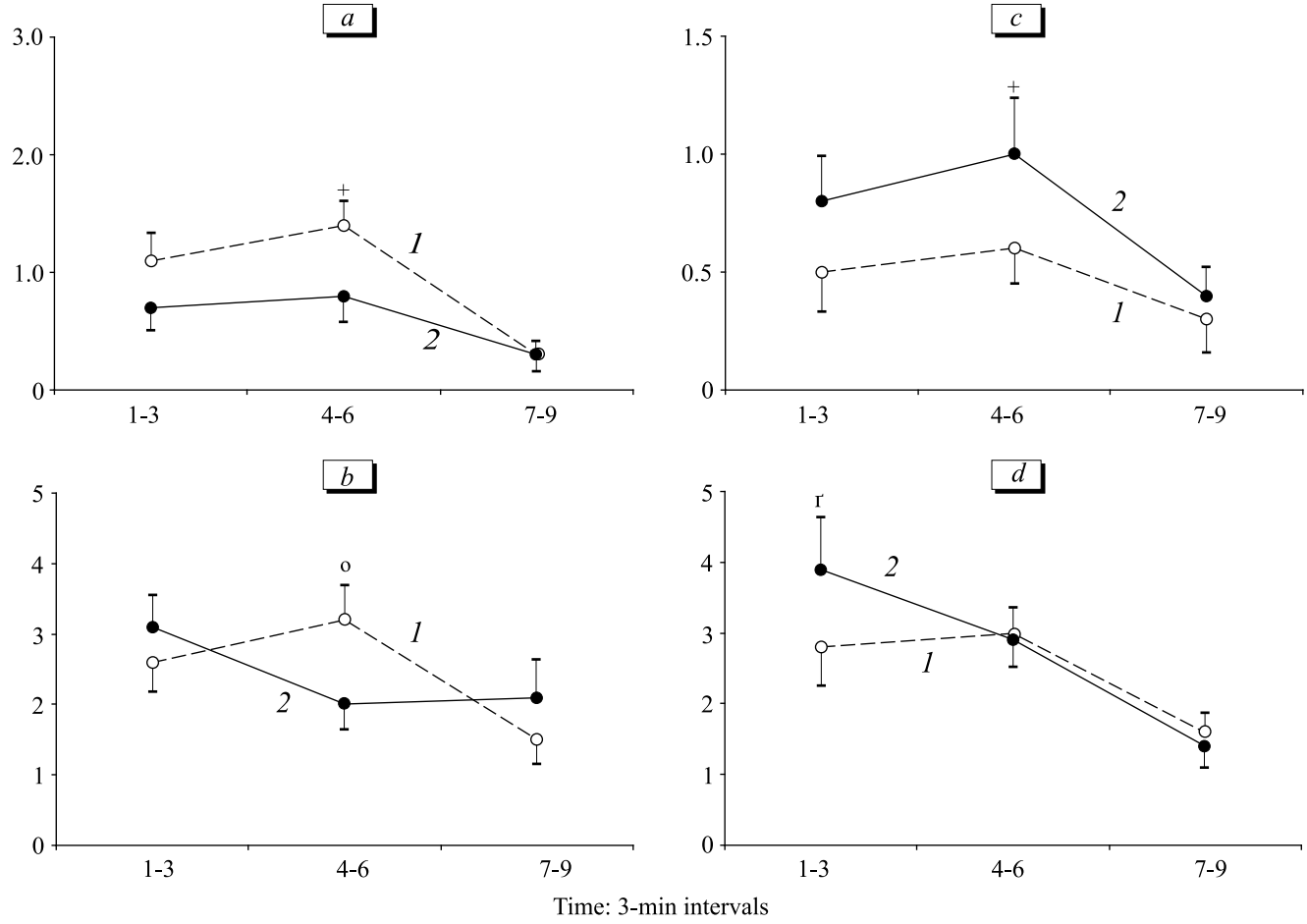

Fig. 1. Effect of analog to N-terminal nociceptin fragment (5 mg/kg intraperitoneally) on open-field behavior of mature rats under dim red and bright white light. *a*) running length (number of crossed segments); *b*) number of visits to arena center; *c*) number of departures from arena wall; *d*) number of explored holes (m±SEM). *1*) control group (n=28), *2*) experimental group (n=31). Intergroup difference at p<0.05 by *Student's and $^+\chi^2$ tests.

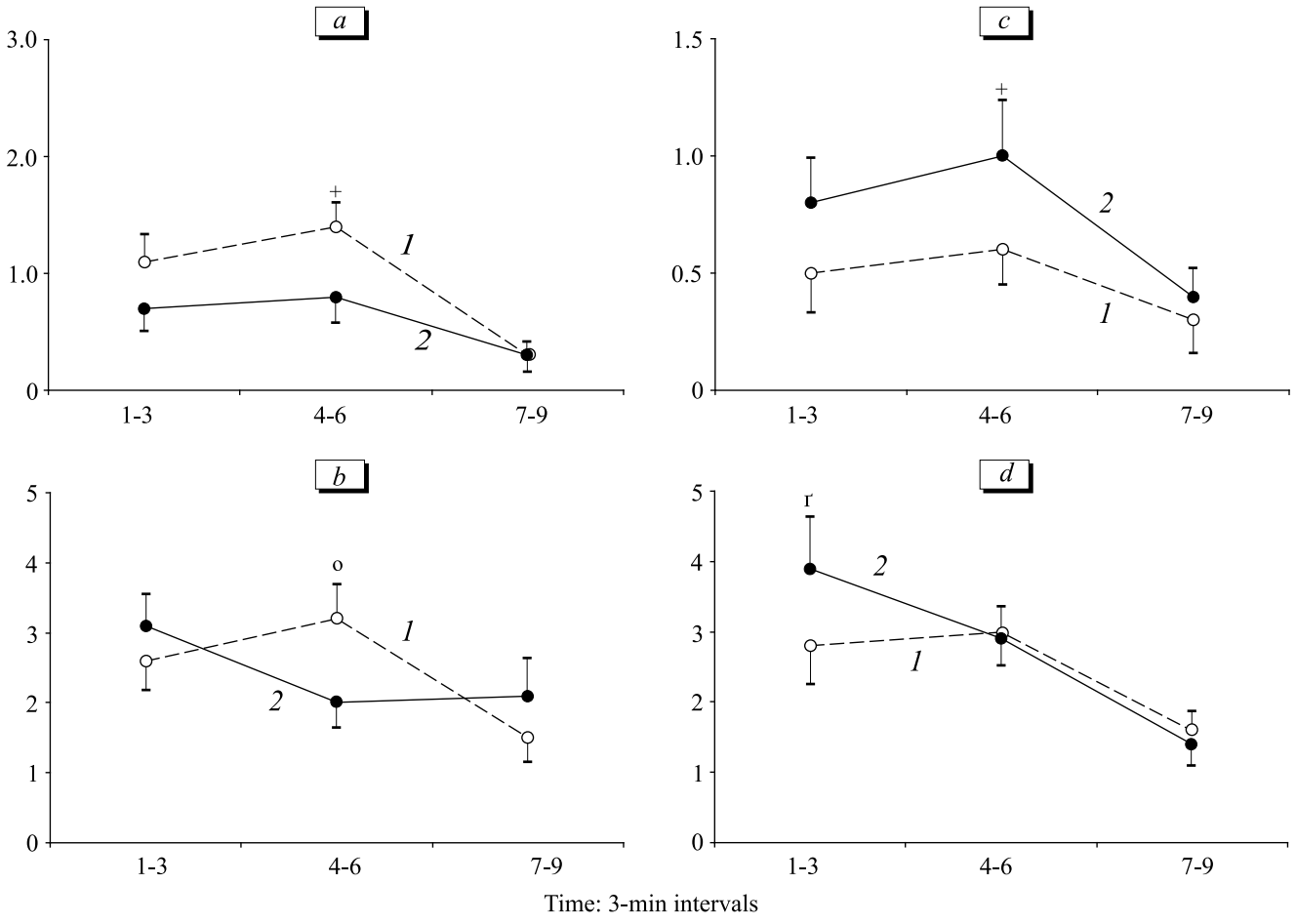

Fig. 2. Effect of analog to N-terminal nociceptin fragment (5 mg/kg intraperitoneally) on open-field behavior of 21- and 42-day rat pups under dim red and bright white light. *a*) number of departures from arena wall in the group of 21-day pups; *b*) number of explored holes in 21-day pups; *c*) number of visits to arena center in 42-day pups; *d*) number of explored hole in 42-day pups. *1*) control group (n=28), *2*) experimental group (n=27). Intergroup difference at $p<0.05$ by °ANOVA, $^{+}\chi^2$ test, and Student's test (only on the second test minute).

phenomena are rather similar to those provoked by the opioid receptor agonists such as endorphins and exorphins [3,5,15]. It can be concluded that the test analog of N-terminal nociceptin fragment under conditions of systemic administration exhibits properties of a functional synergist of opioid transmitters at least in the mature animals. However, the development of its effects during ontogeny is biphasic, which is in striking contrast with other opioids such as β-casomorphins [2].

Further studies of neurotropic activity of N-terminal nociceptin fragment should be performed with a larger set of instrumental methods employing a modified peptide analog with more stable structure. Hopefully, such approach will prompt the researchers to focus on nociceptin fragments as the agents that are promising not only from the viewpoint of their effects on pain perception and on the development of opioid dependency [7], but also from the vista of their possible effects on the balance of explorative activity and anxiety.

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