# Role of the Brain Dopaminergic and Opioid System in the Regulation of "Child's" (Maternal Bonding) Behavior of Newborn Albino Rats

**I. S. Stovolosov, V. A. Dubynin, and A. A. Kamensky**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 9, pp. 248-252, September, 2010
Original article submitted December 23, 2009

Administration of $D_2$ receptor antagonist clebopride in a dose not affecting locomotor activity was followed by a decrease in maternal bonding behavior of 10-day-old and 15-day-old albino rat pups. $D_1$ receptor antagonist SCH23390 had a stimulatory effect only on the behavior of 10-day-old newborns. Opioid peptide β-casomorphin-7 abolished the effect of clebopride in rat pups of the older age group.

**Key Words:** *maternal bonding behavior of newborns; $D_1$ receptor antagonists and $D_2$ receptor antagonists; β-casomorphins*

Aspiration to be in contact with the mother is one of the major motivations of newborn mammals. This contact plays a role of reinforcement. It is not related to the fact that the female serves as a source of food and heat for the young. Differences in the attachment of newborns are associated with various reasons, including the degree of maternal care [9]. The neurochemical basis of "child's" (maternal bonding) behavior is provided by oxytocin, opioid peptides, and catecholamines [10,13]. Opioid receptor agonists (morphine and β-casomorphins) and antagonists increase and decrease the corresponding reactions, respectively [1,3]. μ-Opioid receptor-knockout newborn mice practically do not respond to maternal odor [7].

Here we studied the effect of selective dopamine receptor ligands clebopride ($D_2$ receptor antagonist) and SCH23390 ($D_1$ receptor antagonist) in low doses on the "child's" behavior of newborn rats. In a special series, clebopride and SCH23390 were administered simultaneously to evaluate the interaction of the brain dopaminergic and opioid systems.

Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow State University, Russia. ***Address for correspondence:*** dva-msu@yandex.ru. V. A. Dubynin

## MATERIALS AND METHODS

Experiments were performed on outbred albino rats. The animals aging 10 or 15 days were obtained from the vivarium of the Department of Human and Animal Physiology (Biological Faculty, M. V. Lomonosov Moscow State University). The behavior of rats was studied in a square area (60×60 cm). A sleeping (anesthetized) female was placed into the center of this area. Each pup of the litter was tested twice (before and after drug treatment). The newborn was put thrice ("3 attempts" at 10-sec intervals) to the back of the mother (lying on the side). The distance from the newborn to the tail and head of the mother was similar. The following parameters were evaluated visually in each attempt over 1 min: latency of semi-encirclement (crawling to the ear or tail of the female); climbing (number of attempts to climb the maternal body); number of episodes when the pup pushed the female; period of interaction (total time when the pup was above the female); and period of withdrawal (total time when the pup was not in contact with the female). The degree of maternal bonding behavior (DMBB) was estimated from the results of these tests. The integral criterion was evaluated from five typical reactions of the newborn (Table 1). This approach al-

0007-4888/11/1503-0281 

lows us to reduce individual behavioral variability of rat pups [2]. DMBB scores in 3 attempts were summarized. The results of the 1st (baseline) and 2nd tests (after injection of the preparation or solvent) were compared. Between-group differences in DMBB score were estimated.

Each litter was divided into the treatment group and control group. The animals received an intraperitoneal injection of the preparation or solvent, respectively, 20 min before test 2. Several litters were combined in each series. The mean number of animals per group was 30. The results were analyzed by standard statistical methods (parametric and nonparametric tests).

## RESULTS

The "child's" behavior mainly depends on overall activity of the newborn. A special series was performed to study locomotor activity of newborns. The latency of crawling out of the circle (5 cm in radius) was recorded for 10-day-old rat pups. Locomotor activity of 15-day-old pups was studied in the open field (round area, 40 cm in diameter). A further study of the "child's" behavior was performed with the subthreshold dose (relative to the effect on locomotor activity). The subthreshold doses of $D_2$ receptor antagonist clebopride were 0.5 and 0.1 mg/kg (on days 10 and 15, respectively). The subthreshold dose of $D_1$ receptor antagonist SCH23390 was similar for 10-day-old and 15-day-old rat pups (0.01 mg/kg). The dose of β-casomorphin-7 (5 mg/kg) was selected from the results of our previous experiments [1].

Test 1 (before treatment with the preparation) showed that 10-day-old pups ($n$=198) perform the semi-encircling movement around the mother over 32.8±1.4 sec. The total time of withdrawal was 20.5±2.2 sec. The time of interaction was 8.0±1.1 sec. The number of pushing episodes was 4.7±0.3. The number of climbing reactions was 2.2±0.2. The corresponding parameters for 15-day-old pups ($n$=194) were 20.7±1.1, 19.6±2.1, and 17.7±1.6 sec, respectively. The mean number of pushing episodes and climbing reactions were 2.5±0.2 and 2.8±0.1, respectively.

Treatment of 10-day-old pups with clebopride in a dose not affecting locomotor activity was followed by a decrease in the maternal bonding behavior. The latency of semi-encirclement in treated newborns was shown to increase by 11.6% after clebopride injection (as compared to the results of test 1). In control animals, this period decreased by 21.3% ($p$<0.05). The measurement of DMBB also showed that $D_2$ receptor antagonist suppressed "child's" behavior. Test 2 showed that DMBB score in rat pups of the control and treatment groups was 11.7±0.5 and 8.4±0.7, respectively ($p$<0.01). Variations in DMBB score after clebopride injection were shown to differ in control and treated specimens (2.1±0.9 and -0.6±0.9, respectively, $p$<0.05; Fig. 1, *a*).

The time of withdrawal in 15-day-old pups increased by 16.3% after clebopride injection. By contrast, this period decreased by 46.9% in control animals ($p$<0.05). The time of interaction decreased by 9.1% in treated animals, but increased by 32.1% in control specimens ($p$<0.05). DMBB in rat pups of the treatment group was much lower than in control specimens ($p$<0.01; Fig. 1, *b*).

The next series showed that $D_1$ receptor antagonist SCH23390 has a stimulatory effect on the "child's" behavior of 10-day-old rat pups. The increase of DMBB was more pronounced in treated animals than in con-

**TABLE 1.** DMBB Score

| Behavioral parameter | | Score | | | | |
|---|---|---|---|---|---|---|
| LT of semi-encirclement | time, sec | 0<t≤5 | 5<t≤10 | 10<t≤30 | 30<t≤60 | 60 |
| | points | 4 | 3 | 2 | 1 | 0 |
| Total time of withdrawal | time, sec | 0<t≤10 | 10<t≤30 | 30<t≤60 | | |
| | points | 0 | -1 | -2 | | |
| Time of interaction | time, sec | 0 | 0<t≤5 | 5<t≤10 | 10<t≤30 | 30<t≤60 |
| | points | 0 | 1 | 2 | 3 | 4 |
| Pushing episodes | number | 0 | 0<n≤5 | 5<n≤10 | 10<n≤15 | >15 |
| | points | 0 | 1 | 2 | 3 | 4 |
| Climbing | number | 0 | 0<n≤3 | 3<n≤6 | 6<n≤9 | >9 |
| | points | 0 | 1 | 2 | 3 | 4 |

**Note.** The score is estimated from the distribution of the following five behavioral parameters: latency of semi-encirclement, total time of withdrawal, time of interaction, number of pushing episodes, and number of climbing episodes. LT, latency; t, time; n, number.

trol specimens ($p<0.05$; Fig. 2, *a*). Differences in the time of interaction were most significant. This period increased by 53.2% in treated animals, but decreased by 7.3% in control specimens ($p<0.05$). However, administration of SCH23390 had little effect on DMBB in 15-day-old pups (Fig. 2, *b*).

Previously, we studied the effect of β-casomorphin-7 (YPFPGPI) on the "child's" behavior. The fragment (60-66) of milk β-casein has properties of a μ-opioid receptor agonist. Administration of this peptide in a dose not affecting locomotor activity was shown to increase maternal bonding. It should be emphasized that the effect of β-casomorphin-7 became more pronounced with an increase in the age of animals [1]. Here we studied whether β-casomorphin-7 can abolish the inhibitory effect of clebopride. β-Casomorphin-7 was injected 10 min after clebopride treatment. Control animals received the solvent.

Despite administration of the peptide, clebopride decreased the maternal bonding behavior of 10-day-old rat pups. The latency of semi-encirclement was shown to increase by 6.5% in rat pups receiving β-casomorphin-7 after treatment with $D_2$ receptor antagonist. This period decreased by 12.1% in control animals ($p<0.05$). DMBB score increased from 6.4±0.8 to 9.1±1.0 after injection of the solvent. As distinct from rat pups of the clebopride group, DMBB score in these animals increased from 7.5±0.9 to 8.3±1.0. However, the increase in this period was more significant in control specimens than in treated animals: the change in DMBB score after and before the injection was 2.7±0.7 and 0.8±1.1 points, respectively ($p<0.05$; Fig. 3, *a*).

After administration of clebopride and β-casomorphin-7 to 15-day-old pups, changes in DMBB were similar in control and treated animals (Fig. 3, *b*). Therefore, this peptide abolishes the adverse effect of a $D_2$ receptor antagonist on the "child's" behavior. Moreover, the number of pushing episodes in control specimens decreased by 50.1% after solvent administration (*vs*. 5.3% in treated animals; $p<0.05$).

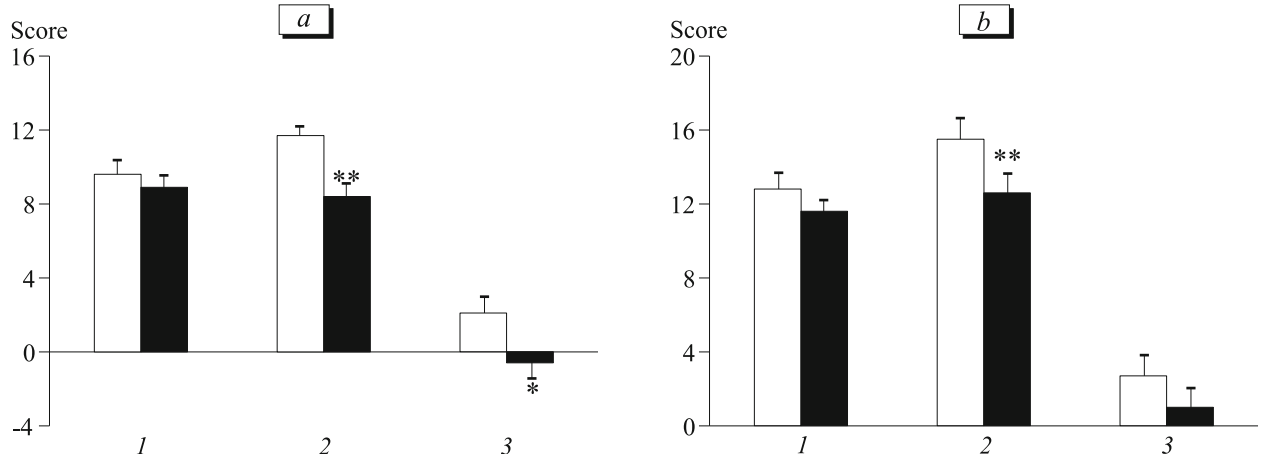


**Fig. 1.** DMBB after intraperitoneal injection of clebopride to 10-day-old (0.5 mg/kg, *a*) and 15-day-old rat pups (0.1 mg/kg, *b*). Here and in Figs. 2 and 3: ordinate, DMBB. Test before injection (*1*); test after injection (*2*); difference in the post-injection and pre-injection parameters (*3*). Light bars, control; dark bars, treatment. *$p<0.05$ and **$p<0.01$ compared to the control.

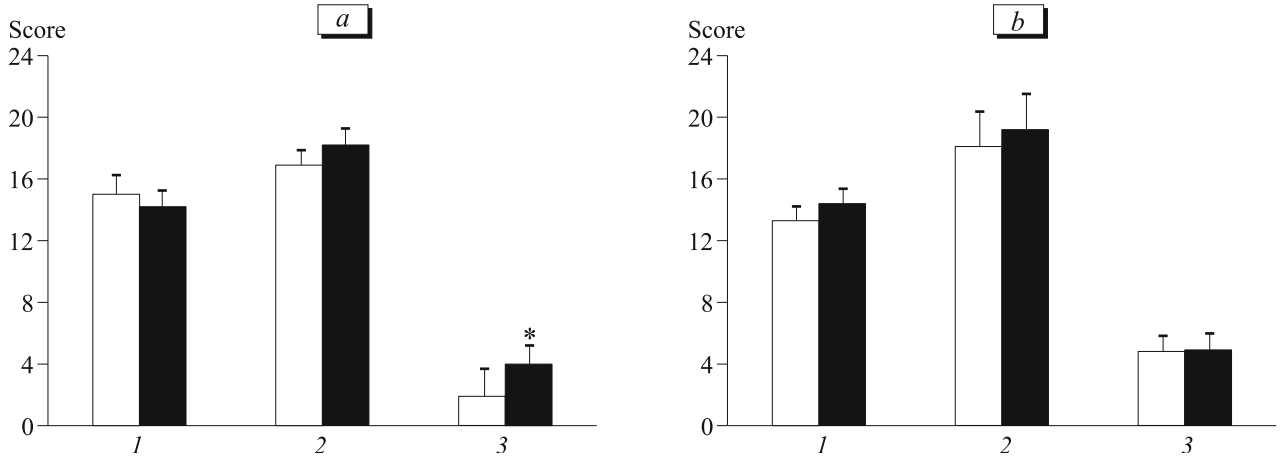


**Fig. 2.** DMBB after intraperitoneal injection of SCH23390 (0.01 mg/kg) to 10-day-old (*a*) and 15-day-old rat pups (*b*).

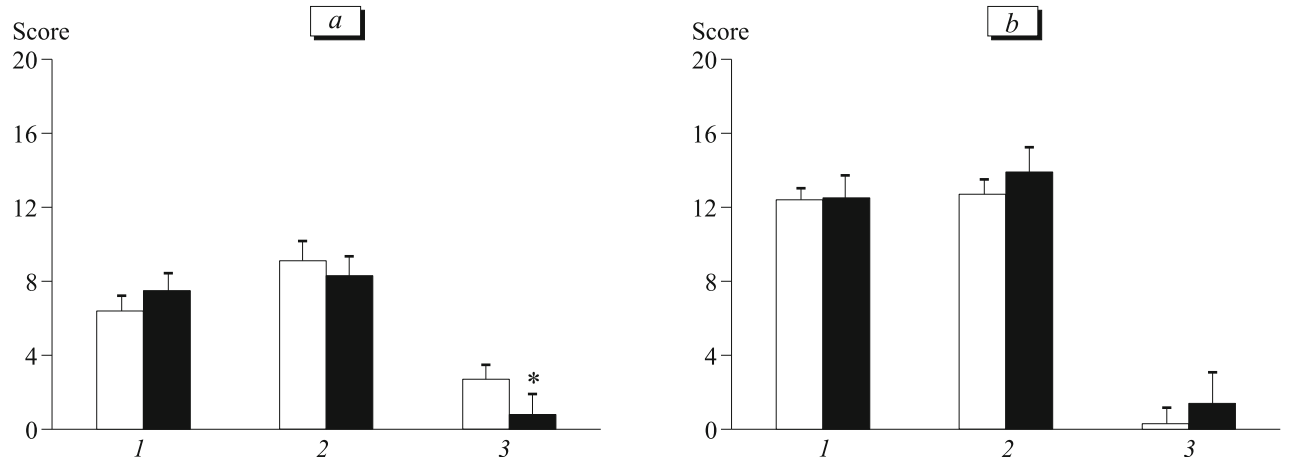


**Fig. 3.** DMBB after intraperitoneal injection of clebopride and β-casomorphin-7 (5 mg/kg) to 10-day-old (*a*) and 15-day-old rat pups (*b*).

The dopaminergic system plays the major role in the interaction of pups and nursing mother. Published data show that administration of $D_2$ receptor agonist quinpirole to the newborn increases the number of sucking episodes with an artificial nipple [4]. Prenatal blockade of dopamine receptors (administration of haloperidol on days 4-15 of pregnancy) is followed by a decrease in ultrasonic vocalization of newborn rut pups during maternal deprivation [5]. Reunion with the mother has an inhibitory effect on ultrasonic vocalization. It should be emphasized that quinpirole potentiates, while $D_2$ receptor antagonist raclopride attenuates this effect [14]. The degree of ultrasonic vocalization under conditions of maternal deprivation increases after administration of SCH23390. The negative effect of clebopride and SCH23390-induced increase in maternal bonding are consistent with published data. The mechanisms of these changes require further investigations. They are probably related to differences in the number and localization of $D_1$ and $D_2$ receptors on forebrain neurons (*e.g.*, in the ventral striatum) [1].

The consequences of clebopride treatment can be considered as a pharmacological model for abnormalities in the "child's" behavior. β-Casomorphin-7 serves as an agent, which compensates for the negative effect of $D_2$ receptor antagonist. This activity of the peptide is observed in 15-day-old pups, which probably results from a progressive maturation of brain μ-opioid receptors in newborn rats [15].

A close relationship exists between the dopaminergic and opioidergic neurotransmitter systems. The reinforcing effect of opioid peptides is often associated with dopamine release [6]. μ-Opioid receptor gene-knockout mice are characterized by reduced pulse activity of dopaminergic neurons [8]. μ-Receptor agonist β-casomorphin-7 probably decreases activity of inhibitory cells that abolish the excitation of dopamine-producing neurons (primarily in VTA) [12]. These changes result in the increased release of dopamine, which compensates for the effect of clebopride.

The study allowed us to obtain new data on the mechanisms of maternal bonding behavior in newborns. We showed that the interaction of the μ-opioidergic and $D_2$-dopaminergic subsystems of the brain plays an important role in this process. These data form the basis for the development of new approaches to pharmacological correction of nervous system activity in newborns ("child's" behavior). Much attention should be paid to dietary supplement with natural β-caseins and constituent opioid fragment. These substances increase the significance of maternal bonding behavior for newborns.

This work was supported by the Russian Foundation for Basic Research (grant No. 08-04-01386).

## REFERENCES

1. V. A. Dubynin, Yu. A. Ivleva, I. S. Stovolosov, *et al.*, *Dokl. Akad. Nauk*, **412**, No. 2, 279-282 (2007).
2. I. S. Stovolosov, V. A. Dubynin, and A. A. Kamensky, *Zh. Vyssh. Nervn. Deyat.*, **60**, No. 3, 5-18 (2010).
3. A. Agmo, S. Barreau, and V. Lemaire, *Dev. Neurosci.*, **19**, No. 6, 505-520 (1997).
4. L. A. Becker and W. P. Smotherman, *Dev. Psychobiol.*, **29**, No. 2, 139-156 (1996).
5. R. Cagiano, R. J. Barfield, N. R. White, *et al.*, *Eur. J. Pharmacol.*, **157**, No. 1, 45-50 (1988).
6. K. C. Lan, A. C. Chang, S. H. Liu, *et al.*, *J. Neurochem.*, 109, No. 2, 382-392 (2009).
7. J. F. Leckman and A. E. Herman, *Biol. Psychiatry*, **51**, No. 1, 27-43 (2002).
8. D. S. Mathon, G. M. Ramakers, J. E. Pintar, and M. Marinelli, *Eur. J. Neurosci.*, **21**, No. 10, 2883-2886 (2005).
9. J. L. Menard and R. M. Hakvoort, *Behav. Brain Res.*, **176**, No. 2, 302-313 (2007).
10. S. Moriceau and R. M. Sullivan, *Dev. Psychobiol.*, **47**, No. 3, 230-242 (2005).

11. J. M. Muller, H. Moore, M. M. Myers, and H. N. Shair, *Behav. Neurosci*., **122**, No. 1, 119-128 (2008).
12. M. Narita, S. Ozaki, and T. Suzuki, *Jpn. J. Pharmacol*., **89**, No. 3, 224-228 (2002).
13. E. E. Nelson and J. Panksepp, *Neurosci. Biobehav. Rev*., **22**, No. 3, 437-452 (1998).
14. H. N. Shair, J. M. Muller, and H. Moore, *Dev. Psychobiol*., **51**, No. 2, 131-146 (2009).
15. J. W. Spain, B. L. Roth, and C. J. Coscia, *J. Neurosci*., **5**, No. 3, 584-588 (1985).