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Dose-Dependent Effects of β-Phenylglutamic Acid Hydrochloride (RGPU-135, Neuroglutam) on Animal Behavior

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β-Phenylglutamic acid hydrochloride (RGPU-135, neuroglutam) in doses of 13-650 mg/kg suppressed depressive behavior of animals in the Porsolt test (*i.e.* produced antidepressant properties), reduced anxiety in the open-field, elevated plus maze, and Vogel conflict tests (*i.e.* produced anxiolytic effects). RGPU-135 in doses of 26-130 mg/kg exhibited more pronounced antidepressant action and in doses of 26 and 52 mg/kg had more pronounced anxiolytic effects. RGPU-135 in doses of 13-78 mg/kg increased locomotor and exploratory activity of animals in the open-field test. Activating effects of this agent decreased with increasing the dose. RGPU-135 in the subtoxic dose (650 mg/kg) suppressed locomotor activity of animals (produced sedative effect).

Key Words: *β-phenylglutamic acid hydrochloride; behavior, dose-effect; glutamic acid; psychotropic effect*

β-Phenylglutamic acid hydrochloride (laboratory code RGPU-135, glutaron, neuroglutam) exhibited a wide spectrum of psychotropic effects: antidepressant, anxiolytic, neuroprotective actions in combination with nootropic, activation, and immunomodulatory properties [1,5-7]. This substance is characterized by low toxicity and potentially high safety [2,3] and therefore is a promising substance for clinical practice. At the stage of preclinical testing, possible relationships between the psychotropic effects of RGPU-135 and its dose should be studied.

Here we studied the dose dependence of the effects of β-phenylglutamic acid hydrochloride on animal behavior.

MATERIALS AND METHODS

The experiments were approved by the Regional Independent Ethic Committee of Volgograd Medical Research Center (protocol No. 140-2011, July 11, 2011) and conducted in accordance with GOST R 53434-2009 Principles of Appropriate Laboratory Practice and European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). The experiments were performed on 56 outbred male rats weighing 180-220 g (8 rats per experimental point; Breeding Center Rappolovo, Russian Academy of Medical Sciences). The animals were kept under standard vivarium conditions with natural light-dark regimen, 20±2°C, 60% humidity, and free access to water and complete granulated feed (GOST R 50258-92).

The doses of RGPU-135 for the study of potential dose-dependent effects on animal behavior were chosen in accordance to the data on acute toxicity and our previous results [2,3,5]. Experimentally proved therapeutic dose of the agent 26 mg/kg constituting $^1/_{300}$ LD_{50} [3,5] served as a basic dose and other doses used in our study were multiple of this dose: 13 mg/kg ($^1/_{600}$ LD_{50}), 52 mg/kg ($^1/_{150}$ LD_{50}), 78 mg/kg ($^1/_{100}$ LD_{50}), and 130 mg/kg ($^1/_{60}$ LD_{50}). The dose of 650 mg/kg (25-fold surpassing the therapeutic dose, $^1/_{10}$ LD_{50}) that can be considered as the subtoxic dose was also used in the experiment. Animal behavior was evaluated in psychopharmacological tests [4]: Porsolt

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forced swimming test, open-field test, elevated plus maze, and Vogel conflict test. RGPU-135 in study doses was administrated intragastrically in 2% starch mucilage 60 min before the beginning of experiments. Control animals received same volume of 2% starch mucilage.

Statistical analysis of obtained data was performed using Statistica 6.0 and BioStat 2008 software and rank univariate Kruskal–Wallis test and Newman–Keuls criterion.

RESULTS

RGPU-135 in doses of 26-130 mg/kg decreased the severity of depressive behavior in animals in the Porsolt forced swimming test. Under these conditions, the duration of immobility significantly decreased (Fig. 1, *b*), the latent period (LP) of immobility (Fig. 1, *a*), number of jumps (Fig. 1, *c*), and duration of active swimming (Fig. 1, *d*) increased. These data reflect antidepressant properties of RGPU-135. The most pronounced antidepressant effect was observed after administration of RGPU-135 in a dose of 26 mg/kg. Increasing the dose was followed by weakening of the antidepressant effect. RGPU-135 in a dose of 13 mg/kg did not affect LP of immobility, but significantly reduced its duration and increased the parameters of active avoidance (number of jumps, and duration of active swimming), which attests to primarily activating effect of this dose. The subtoxic dose of the agent (650 mg/kg) also produced a slight antidepressant effect, which was seen from significantly reduced duration of immobility; however, the decrease in the number of jumps observed in these animals can reflect its sedative effect. According to the results of Porsolt test, the antidepressant effect of neuroglutam in the studied doses decreased in the following order: 26>52>78=130>13>650 mg/kg.

In the open-field test, RGPU-135 in doses 3>26>52>78 mg/kg produced activating effect and increased spontaneous motor (Fig. 2, *a*) and exploratory (Fig. 2, *b*) activities. The agent in a dose of 13 mg/kg had the most pronounced activating effect: motor activity of animals received this dose was significantly higher than after administration of other doses. Administra-

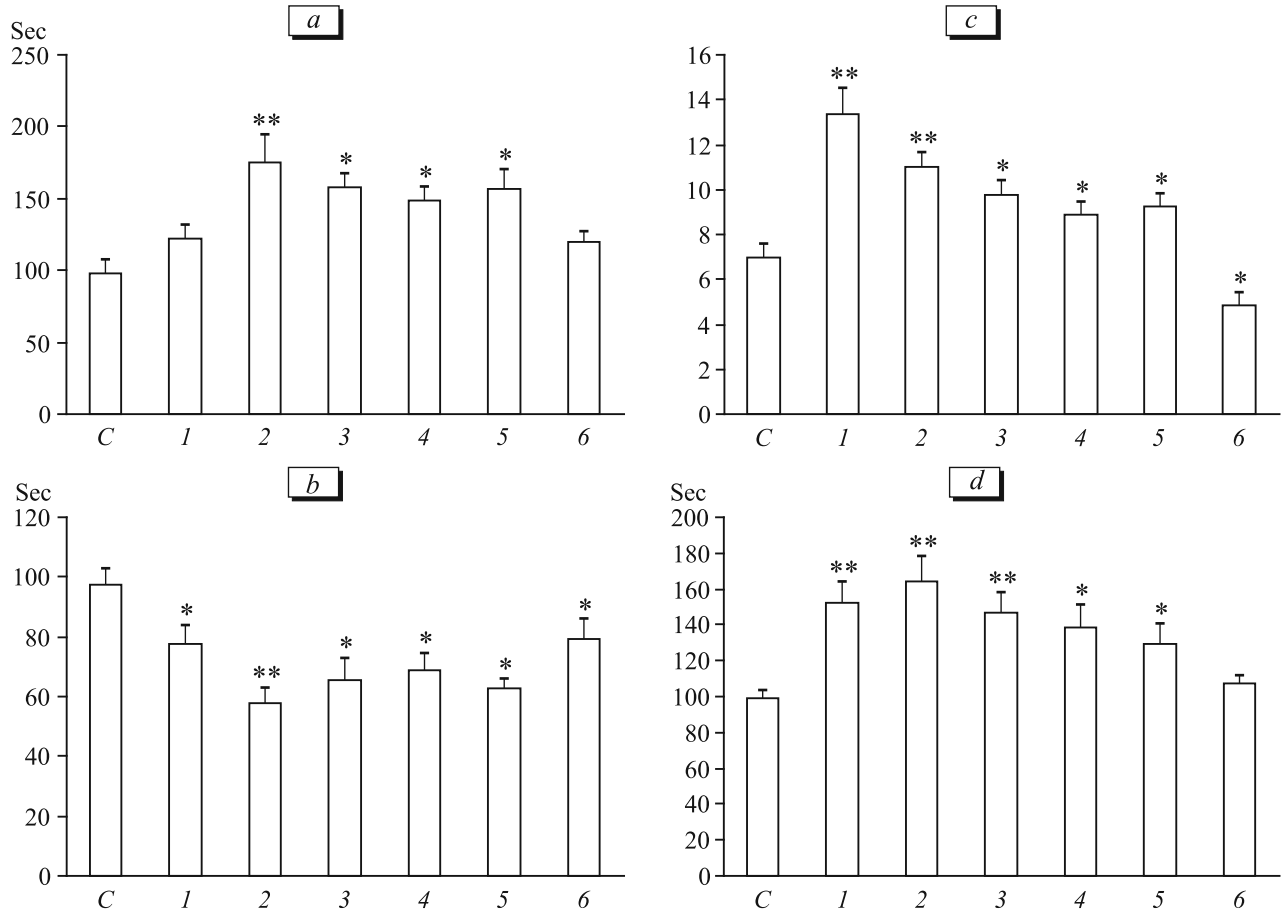

Fig. 1. Effects of RGPU-135 on depression-like behavior of animals in the Porsolt forced swimming test. *a*) LP of immobilization; *b*) total duration of immobilization; *c*) number of jumps; *d*) total duration of active swimming. Here and in Figs. 2 and 3: *C*, control; *1*) 13 mg/kg; *2*) 26 mg/kg; *3*) 52 mg/kg; *4*) 78 mg/kg; *5*) 130 mg/kg; *6*) 650 mg/kg. **p*<0.05, ***p*<0.01 in comparison with the control.

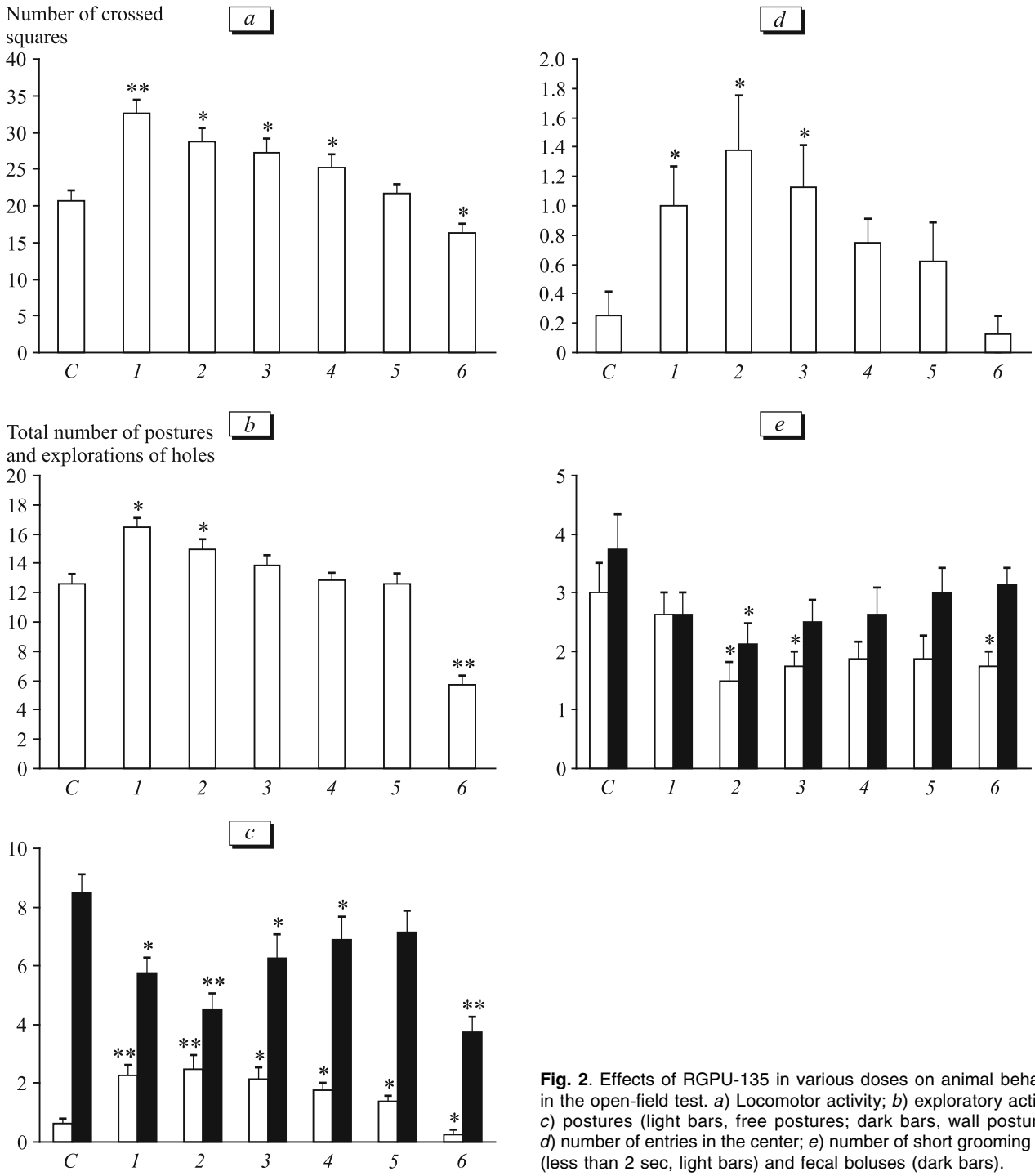

Fig. 2. Effects of RGPU-135 in various doses on animal behavior in the open-field test. *a*) Locomotor activity; *b*) exploratory activity; *c*) postures (light bars, free postures; dark bars, wall postures); *d*) number of entries in the center; *e*) number of short grooming acts (less than 2 sec, light bars) and fecal boluses (dark bars).

tion of RGPU-135 in the subtoxic dose (650 mg/kg) was followed by a decrease in motor and exploratory activity, which attests to sedation and is consistent with the results of Porsolt test.

RGPU-135 in doses of 13-78 mg/kg produced anxiolytic effects in the open-field test. The number of "anxious" wall postures significantly decreased, and the number of "calm" free postures increased (Fig. 2, *c*). In addition, administration of RGPU-135 in doses of 26 and 52 mg/kg significantly increased the number of entries to the center and reduced the number of abortive grooming, which confirm the anxiolytic effect (Fig. 2, *d*, *e*). RGPU-135 in a dose of 26 mg/kg significantly decreased the number of fecal boluses (autonomic sign of anxiety). Anxiolytic activity of the agent decreased with increasing the dose: after administration of RGPU-135 in a dose of 130 mg/kg, only a tendency to anxiolytic effect was observed (the num-

TABLE 1. Effects of RGPU-135 in Various Doses on Animal Behavior in the Vogel Conflict Test

Dose, mg/kg	LP of first punished approach to the bottle, sec	Number of punished approaches to the bottle
Control	75.50±9.79	2.75±0.37
13	54.88±5.39	3.50±0.27
26	39.00±6.28**	4.75±0.53**
52	45.50±3.91*	4.00±0.38*
78	49.25±6.29*	3.75±0.36
130	62.25±5.83	3.50±0.57
650	94.38±7.52	1.88±0.44

Note. *p<0.05, **p<0.01 in comparison with the control.

ber of free postures significantly increased). The number of entries to the center increased in animals receiving RGPU-135 in a dose of 13 mg/kg, but autonomic signs of anxiety did not change and locomotor activity increased. These changes can be a result of the activating effect of the substance in this dose. Administration of RGPU-135 in a dose of 650 mg/kg was followed by a significant decrease in the number of wall and free postures and reduction of locomotor and exploratory activity, which can be related to the sedative effect of the agent. Thus, the anxiolytic effect of RGPU-135 in the studied doses in the open-field test decreased in the following order: 26>52>78>13>650>130 mg/kg.

RGPU-135 in all studied doses produced anxiolytic effects on animal behavior in the elevated plus maze: the time spent in the open arms of the maze significantly increased (Fig. 3, *a*). The most pronounced anxiolytic effect was observed after administration of RGPU-135 in doses of 26-130 mg/kg: the number of entrances, head-dipping postures, and rearing postures in the open arms significantly increased (Fig. 3, *b*-*d*). Activity of RGPU-135 in doses of 26 and 52 mg/kg was more pronounced than in other doses. Increasing the dose led to a decrease in the anxiolytic effects of RGPU-135. The increase in the number of entrances into open arms was significantly higher and the time spent in the open arms and number of rearing postures was slightly higher in animals receiving RGPU-135 in a dose of 13 mg/kg than in specimens treated by this agent in a dose of 26 mg/kg (Fig. 3 *a*, *b*, *d*). These data

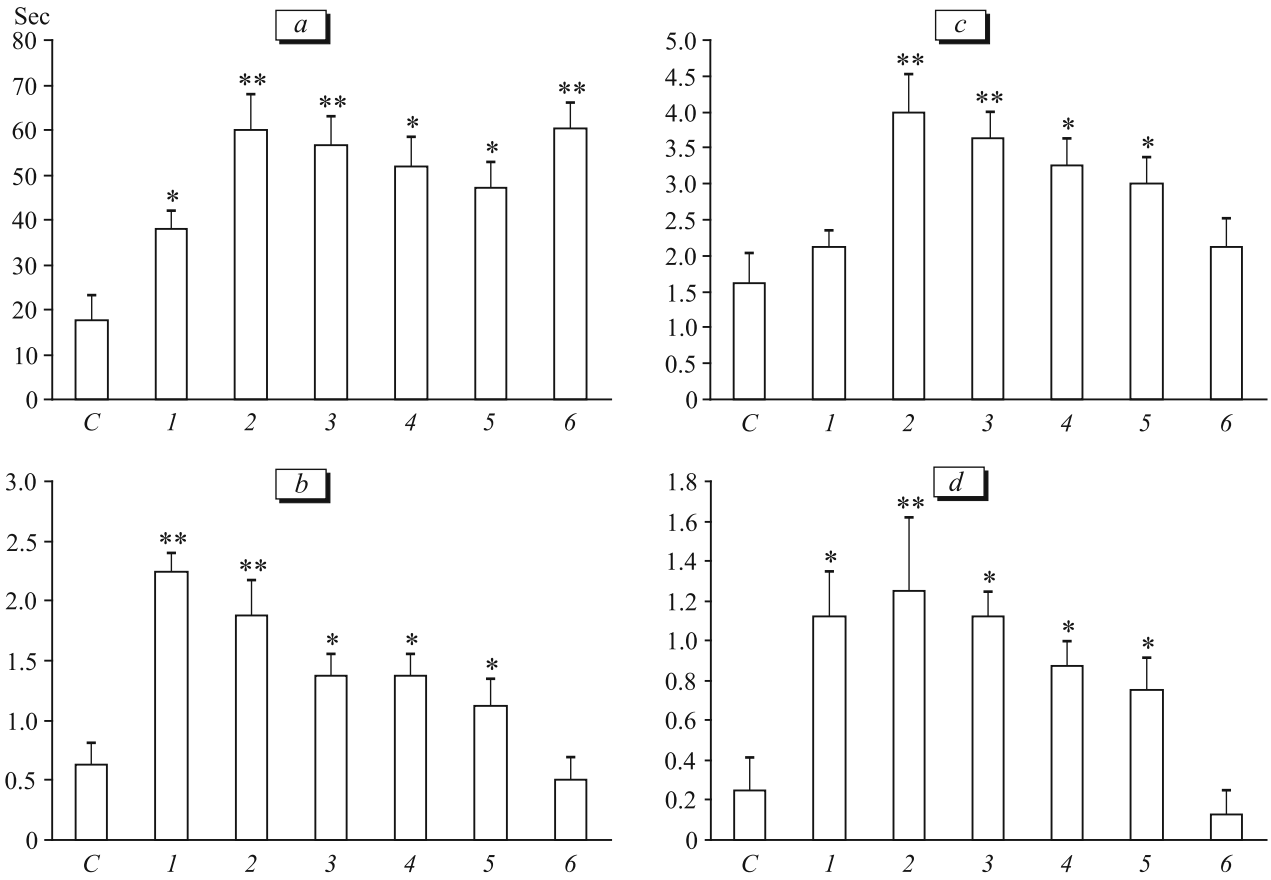

Fig. 3. Effects of RGPU-135 in various doses on animal behavior in the elevated plus maze. *a*) Time spent in the open arms; *b*) number of entries in the open arms; *c*) number of head-dipping postures; *d*) postures in the open arms.

suggest that activating effect is more pronounced than anxiolytic. RGPU-135 in a dose of 650 mg/kg significantly prolonged the time spent in the open arms, but did not affect other parameters. All these data attest to anxiolytic and sedative effects of the agent in this dose.

RGPU-135 in doses of 26-78 mg/kg exhibited anxiolytic properties in the Vogel conflict test: it significantly decreased LP of first punished approach to a bottle with water and increased the number of punished drinks (Table 1). The anxiolytic effect of RGPU-135 in studied doses decreased in the following order: 26>52>78 mg/kg. This substance in doses of 13 and 130 mg/kg exhibited no anxiolytic effect in the conflict situation. LP of first punished approach tended to increase and the number of punished approaches to the bottle tended to decrease after treatment of animals with RGPU-135 in a dose of 650 mg/kg. These changes can be a result of sedative effect of neuroglutam in this dose.

It was shown that RGPU-135 reveals antidepressant properties in all study doses: from low therapeutic dose (13 mg/kg) to subtoxic dose (650 mg/kg). The most pronounced effect was found after treatment with RGPU-135 in moderate and high therapeutic doses (26-130 mg/kg). We also found anxiolytic properties of this agent in all study doses, and the affect was most pronounced after treatment with the agent in lower doses (26 and 52 mg/kg). The activating effect was maximum after administration of RGPU-135 in the low concentrations (13 and 26 mg/kg). Increase the dose to the subtoxic (650 mg/kg) led to inversion of the pharmacological effect (from activation to sedation), which, according to previous data, can be related to the toxic effect manifested in symptoms of CNS deprivation [2,3]. Thus, RGPU-135 in low and moderate therapeutic doses (13 and 26 mg/kg) produces significant antidepressant, anxiolytic, and activating effects. These properties are less pronounced after administration of RGPU-135 in doses of 52 and 78 mg/kg. RGPU-135 in high therapeutic dose (130 mg/kg) reveals moderate antidepressant and anxiolytic effects and in subtoxic dose reveals mild antidepressant and moderate anxiolytic properties.

β-Phenylglutamic acid hydrochloride (RGPU-135, neuroglutam) in all studied doses (13-650 mg/kg) suppresses depression-like behavior in the Porsolt forced swimming test (antidepressant effects), and decreases anxiety in the open field test, elevated plus maze, and Vogel conflict test (anxiolytic effects). Antidepressant effect is the most pronounced after administration of RGPU-135 in doses of 26-130 mg/kg, and anxiolytic effect is the most pronounced after administration of RGPU-135 in doses of 26 and 52 mg/kg. RGPU-135 in doses of 13-78 mg/kg increases locomotor and exploratory activity in the open-field test (activating effect). This effect is the most pronounced after treatment with the agent in doses of 13 and 26 mg/kg, and reduces when the dose rises. RGPU-135 in the subtoxic dose of 650 mg/kg suppresses motor and exploratory activity in animals (sedative effect).

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