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The effects of kappa opioid agonist RU-1205 on local field potentials and behavior in the discriminative stimulus paradigm

K.Yu. Kalitin[1, 2], O.Yu. Mukha[1, 2], A.A. Spasov[1, 2]

[1] Volgograd State Medical University,
1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131
[2] Scientific Center for Innovative Drugs,
39, Novorossiyskaya Str., Volgograd, Russia, 400087

E-mail: olay.myha14@gmail.com

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Kappa opioid receptors play a pivotal role in regulating both physiological and cognitive processes. RU-1205, a benzimidazole derivative acting as a specific kappa opioid receptor agonist, has demonstrated the capacity to modulate neuronal activity. However, the nuanced effects of RU-1205 on neuronal activity remain incompletely understood.

The aim of the study was to identify and elucidate the effects of the kappa opioid agonist RU-1205 on local field potentials and behavior in the discriminative stimulus paradigm.

Materials and methods. The experiments were conducted in male rats weighing 260–280 g. The animals were surgically implanted with cortical electrodes (F — frontal, O — occipital, P — parietal) as well as deep electrodes in specific brain regions, including the medial prefrontal cortex (mPFC), hippocampus (Hipp), nucleus accumbens (NAc), ventral tegmental area (VTA) and amygdala (Amy). LFP signals were obtained and analyzed after the administration of the compound RU-1205 (350 µg/5 µl intracerebroventricular injections) using spectral and coherence analysis methods. Drug discrimination paradigm was employed to evaluate the similarity of the compound RU-1205 to the selective kappa opioid receptor agonist U-50488 and the p38 MAPK inhibitor SB203580 (including in combination with the opioid receptor blocker naloxone).

Results. Electrophysiological changes observed include an increase in power of theta frequencies (4–8 Hz) in F, P and mPFC leads, along with a reduced power of delta frequencies (0.5–4 Hz) in O and Hipp)leads, and a suppression of gamma activity (30–50 Hz) in F and mPFC leads, all with statistical significance (p <0.05). Post-administration of RU-1205 resulted in a decreased coherence between pairs of electrodes: P–O, P–F, F–O, and mPFC–Hipp (all p <0.05). It was found that the compound RU-1205 is similar to U-50488 and does not exhibit p38 inhibitory activity in the discriminative stimulus paradigm.

Conclusion. Compared to the selective kappa-opioid agonist U-50488, the compound RU-1205 induces less significant changes in LFP activity without electrophysiological and behavioral signs of beta-arrestin pathway activation. The overall data suggest that RU-1205 is a functionally selective agonist of kappa-opioid receptors.

Keywords*:* kappa opioid receptors; RU-1205; electrophysiology; brain bioelectrical activity; spectral analysis; coherence analysis; discriminative stimulus paradigm; drug discrimination

Abbreviations: CNS — central nervous system; ACSF — artificial cerebrospinal fluid; BLA — basolateral amygdala; dHPC — dorsal hippocampus; Hipp — hippocampus; LFP — local field potential; NAc — nucleus accumbens; p38 MAPK — p38 mitogen-activated protein kinase; PrL — prelimbic cortex; VTA — ventral tegmental area.

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Влияние каппа-опиоидного агониста РУ-1205 на локальный полевой потенциал и поведение в модели дискриминации стимула

К.Ю. Калитин[1, 2], О.Ю. Муха[1, 2], А.А. Спасов[1, 2]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации, Россия, 400131, г. Волгоград, пл. Павших Борцов, д. 1
[2] Научный центр инновационных лекарственных средств федерального государственного бюджетного образовательного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации, Россия, 400087, г. Волгоград, ул. Новороссийская, д. 39

E-mail: olay.myha14@gmail.com

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Каппа-опиоидные рецепторы играют ключевую роль в регуляции физиологических и психических процессов. Было показано, что производное бензимидазола РУ-1205, специфический агонист каппа-опиоидных рецепторов, модулирует активность нейронов. Однако комплексное воздействие соединения РУ-1205 на нейрональную активность остается недостаточно изученным.

Цель. Поиск и интерпретация изменений локального полевого потенциала, а также оценка поведения в модели дискриминации стимула под влиянием каппа-опиоидного агониста РУ-1205.

Материалы и методы. Крысам (260–280 г) имплантировали корковые (F — фронтальные, O — окципитальные, P — париетальные), а также глубокие электроды в зону медиальной префронтальной коры (mPFC), гиппокампа (Hipp), прилежащего ядра (NAc), вентральной области покрышки (VTA) и миндалины (Amy). Проводился спектральный и когерентный анализ LFP-сигналов, полученных после введения соединения РУ-1205 (350 мкг/5 мкл интрацеребровентрикулярно). Использовалась модель дискриминации стимула, чтобы оценить сходство соединения РУ-1205 с селективным агонистом каппа-опиоидных рецепторов U-50488 и ингибитором MAPK p38 SB203580 (в том числе в комбинации с блокатором опиоидных рецепторов налоксоном).

Результаты. Зафиксированы изменения: повышение мощности в диапазоне тета-частот (4–8 Гц) на отведениях F, P и mPFC, снижение мощности в диапазоне дельта-частот (0,5–4 Гц) сигналов с O и Hipp отведений, а также подавление гамма-активности (30–50 Гц) на отведениях F и mPFC (p <0,05). После введения РУ-1205 наблюдалось снижение когерентности между парами электродов: P–O, P–F, F–O и mPFC–Hipp (p <0,05). Отсутствие p38-ингибирующей активности РУ-1205 и его сходство с U-50488 подтверждено в модели дискриминации стимула.

Заключение. Установлено, что по сравнению с селективным каппа-опиоидным агонистом U-50488 соединение РУ-1205 вызывает менее выраженные изменения LFP-активности без электрофизиологических и поведенческих признаков активации бета-аррестинового пути. Совокупность данных свидетельствует о принадлежности соединения РУ-1205 к функционально селективным агонистам каппа-опиоидных рецепторов.

Ключевые слова: каппа-опиоидные рецепторы; РУ-1205; электрофизиология; биоэлектрическая активность мозга; спектральный анализ; когерентный анализ; модель дискриминации стимула; дискриминация лекарственных средств

Список сокращений: ЦНС — центральная нервная система; ACSF — искусственная спинномозговая жидкость; BLA — базолатеральная миндалина; dHPC — дорсальный гиппокамп; Hipp — гиппокамп; LFP — локальный полевой потенциал; NAc — прилежащее ядро; p38 MAPK — p38 митоген-активируемая протеинкиназа; PrL — прелимбическая кора; VTA — вентральная область покрышки.

INTRODUCTION

Kappa opioid receptors are involved in the modulation of various physiological and cognitive functions, including pain perception, stress response, and mood modulation [1]. We have previously demonstrated that the benzimidazole derivative RU-1205, a specific kappa-opioid receptor agonist, modulates neuronal activity [2, 3]. However, the full scope of its effects on neuronal activity remains to be fully elucidated.

Local field potentials (LFPs) reflect the collective electrical activity of large neuronal populations and

are a subject of comprehensive investigation in electrophysiology and neuroscience [4]. The analysis of LFP signals provides invaluable information for understanding the neuropharmacological profiles of pharmaceutical drugs and experimental compounds. Furthermore, the use of intracerebral electrodes enables the study of local changes in neuronal bioelectrical activity within target brain regions. Coherence analysis can also be employed to investigate functional connectivity between different brain areas, allowing for a more detailed examination of the interactions between various frequency components of the signal and the identification of complex dependencies that might be overlooked in spectral analysis alone [5].

Several studies have reported a pronounced influence of kappa-opioid agonists on brain bioelectrical activity. Following the administration of salvinorin-A, an increase in the power of delta (1.3–3.5 Hz) and gamma (35–40 Hz) waves, accompanied by a decrease in alpha wave power (7.5–13 Hz), was observed on the electroencephalogram [6]. A study utilizing a *Salvia divinorum* Epling & Játiva extract revealed ECoG changes characterised by an increased spectral power density in signals from frontal leads and a decreased power in signals from occipital leads [7]. The kappa-opioid agonists enadoline and PD117302 induced a dose-dependent shift in EEG power, particularly within the 4 to 8 Hz frequency range. These effects were abolished by norbinaltorphimine, confirming the involvement of kappa-opioid receptors in the observed effects [8].

Key brain regions associated with the analgesic and aversive effects of kappa-opioid agonists include the cerebral cortex (mPFC), hippocampus (Hipp), nucleus accumbens (NAc), ventral tegmental area (VTA), and amygdala (Amy) [9]. These areas exhibit the highest density of kappa-opioid receptors [10] and were therefore selected for this investigation.

The kappa-opioid receptor agonist RU-1205 is of particular interest due to its unique pharmacological profile. The compound does not produce aversive effects, as determined by the conditioned place preference test, nor does it induce tolerance upon chronic administration, distinguishing it from typical kappa-opioid agonists [11, 12]. To explain these properties, a multitarget mechanism of action was hypothesized, suggesting that the effects of RU-1205 may involve not only the activation of kappa-opioid receptors but also an additional inhibitory effect on p38 MAP kinase. This hypothesis was based on previous experiments demonstrating that the aversive effects of kappa-opioid agonists could be completely prevented by the administration of the p38 inhibitor SB203580 [13]. It was also established that RU-1205 could suppress the aversive effects of the kappa-opioid agonist U-50488 [11]. The hypothesis of p38-inhibitory activity can be tested using a drug discrimination paradigm. This methodology is widely employed in psychopharmacology and behavioral science to assess the perceptual and cognitive effects of various chemical substances, based on the principle that animals can be trained to distinguish between the interoceptive effects of different pharmacological agents.

THE AIM of this study was to evaluate the impact of RU-1205 on LFP activity in the cerebral cortex, hippocampus, medial prefrontal cortex, amygdala, nucleus accumbens, and ventral tegmental area. A further aim was to determine whether RU-1205 possesses p38 MAPK-inhibitory properties by assessing the ability of rats to discriminate its effects from those of the p38 inhibitor SB203580.

MATERIALS AND METHODS

Study design

The study comprised two main stages. At the first stage, LFPs were recorded following intracerebroventricular administration of RU-1205, and the resulting signals were subjected to spectral and coherence analyses. In the second stage, we analyzed the discriminative stimulus properties of RU-1205.

Test compounds

The study used 9-(2-morpholinoethyl)-2-(4-fluorophenyl)imidazo[1,2-a]benzimidazole (RU-1205), synthesized at the Research Institute of Physical and Organic Chemistry of the Southern Federal University (RF Patent No. 2413512 C1, purity ≥ 99,46%), U-50488 (Sigma Aldrich, USA), and SB203580 (Sigma Aldrich, USA).

Study duration and conditions

The study was conducted between July and September 2023. All experimental procedures were carried out at the Laboratory of Electrophysiological Research, Scientific Center for Innovative Drugs of the Volgograd State Medical University.

Ethics approval

Animal experiments were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and

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Other Scientific Purposes, the principles of Good Laboratory Practice (GLP) (GOST 33044-2014, 2021), and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The study protocol was approved by the Local Ethics Committee of Volgograd State Medical University (Registration number IRB00005839 IORG0004900, Minutes No. 2022/096, dated January 21, 2022).

Animals

The experiments were conducted on outbred male albino rats (*n* = 41) weighing 260–280 g. The animals were housed under standard vivarium conditions with a 12-hour light-dark cycle and at the temperature of 22 ± 2 °C, a relative humidity of 40–50%, and *ad libitum* access to food and water.

Surgical procedures

Under 2% isoflurane anesthesia (Laboratories Karizoo, S.A., Spain) administered via a rodent gas anesthesia system (Gas Anesthesia System-21100, Ugo Basile, Italy), stainless steel electrodes (0.1 mm diameter), insulated along their entire length except for the tip, were implanted into the right cerebral hemisphere according to the following stereotaxic coordinates relative to bregma:

Cortical electrodes: F — anteroposterior (AP) = 0,00, mediolateral (ML) = +2.00; P — AP = -4.08, ML = +2.00; O — AP = -7.08, ML = +2.00.

Deep electrodes: prelimbic cortex (PrL) — AP = +2.7 mm, ML = +0.8 mm, dorsoventral (DV) = 3.8 mm; basolateral amygdala (BLA) — AP = -2.8 mm, ML = +5–5.3 mm, DV = 8.8 mm; hippocampus (Hipp) — AP = –4.9 mm, ML = +4.8 mm, DV = 6.0 mm; ventral tegmental area (VTA) — AP = -5.2 mm, ML = +1,0 mm, DV = 8.6 mm; nucleus accumbens (NAc) — AP = +1.8 mm, ML = +1.6 mm, DV = 7.3 mm.

For intracerebroventricular injections, a 21-gauge stainless steel guide cannula was implanted into the left lateral ventricle using the following stereotaxic coordinates from bregma: AP = -0.6 mm, ML = -1.6 mm, DV = 4.0 mm. The electrodes and cannula were secured to the skull using dental acrylic (Protacryl-M, Ukraine) and two stainless steel screws.

Postoperatively, animals were housed individually and given 7 days to recover.

Signal recording

For the electrophysiological experiments, animals were assigned to two groups (*n* = 16): 1) the first group (*n* = 8) received an intracerebroventricular (i.c.v.) injection of 5 µl of artificial cerebrospinal fluid (ACSF); 2) the second group (*n* = 8) received RU-1205 at a dose of 350 µg / 5 µl i.c.v., equivalent to the intraperitoneal ED_{80} determined in analgesic activity assays. LFPs were recorded using a laboratory electroencephalograph (NVX-36, MKS, Russia). LFP activity was recorded in a monopolar montage against a common average reference at a sampling rate of 500 Hz. Thirty minutes after i.c.v. administration of the test substance or ACSF, LFPs were recorded for 10 minutes.

Spectral analysis

The signal was filtered using a basic FIR filter with a passband of 0,5 to 50 Hz. Subsequently, independent component analysis was applied to remove muscle artifacts. Spectral analysis was performed via a direct discrete Fourier transform for the following frequency bands: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–50 Hz). The analysis was conducted in Python (v.3.11.3) using the MNE-Python package (v.1.6.1)[1].

Coherence analysis

Magnitude-squared coherence [14] was calculated to quantify phase synchronization in the frequency domain between LFP signals (1-second epochs) for all electrode pairs using the ‘mscohere’ function (parameters: window = 1 s, noverlap, nfft = 500, fs = 500) in MATLAB (R2023b; MathWorks Inc., United States) under an individual license. The analysis was focused on the theta frequency range (4–8 Hz). Fisher’s z-transformation was applied to all coherence values for data normalization to permit parametric statistical testing.

Analysis of the discriminative stimulus properties of RU-1205

The drug discrimination paradigm is used to study the effects and mechanisms of action of pharmacological agents [15–17]. While conditioning can be established using food reinforcement, this typically requires 4–7 weeks of training. To accelerate the conditioning process, electrical stimulation of the ventral tegmental area was employed, which significantly shortened the training period.

For the experiment on the discriminative stimulus properties of RU-1205, intact rats (*n* = 25) were implanted with a stimulating stainless steel electrode (0.1 mm) in the ventral tegmental area using the

[1] Zenodo. MNE-Python (v1.6.1). Available from: https://zenodo.org/records/10519948 2

following stereotaxic coordinates from bregma: AP = -5.2 mm, ML = +1.0 mm, DV = -8.6 mm.

During the initial training phase, animals were placed in an operant chamber with a single available lever (right or left) (Fig. 1). 10 min before the session, animals were injected with either: 1) RU-1205 at a dose of 350 µg / 5 µl i.c.v. (n = 9); 2) a combination of RU-1205 (350 µg) and naloxone (200 µg) in 5 µl ACSF i.c.v. (n = 8); 3) a combination of SB203580 (1 µg) and naloxone (200 µg) in 5 µl ACSF i.c.v. (n = 8); or a control solution of ACSF (5 µl) / naloxone (200 µg). Naloxone was co-administered with SB203580 in the third group to confirm that naloxone itself does not interfere with the discrimination of p38 MAPK-inhibitory activity. Furthermore, its inclusion with RU-1205 in tests against SB203580-trained animals was intended to prevent potential false-negative results, where the kappa-opioid component of RU-1205 might mask its discriminability. For half of the animals in each group, a left lever press was reinforced following control solution administration and a right lever press was reinforced following test compound administration; this was reversed for the other half.

A correct lever press resulted in the delivery of an electrical stimulus (24 biphasic 60 Hz pulses, 2 ms duration, fixed-ratio 1:1) to the reinforcement area via an isolated stimulator (A-M Systems MODEL 4100, USA). The current intensity was individually titrated (80–150 µA) to a level below that which elicited involuntary movements, avoidance behavior, or vocalizations. Over the subsequent 3–4 days, the fixed ratio was gradually increased to 10.

During the discrimination training phase, the fixed ratio was again progressively increased from 1 to 10. Each session lasted 20 min, with both levers available simultaneously. An incorrect lever press reset the response counter, requiring the animal to complete 10 consecutive correct operant actions to receive reinforcement. The administration of ACSF / naloxone or test compounds was randomized across three weekly sessions (Table 1).

The criterion for acquisition of discrimination was defined as an accuracy of ≥ 80% on the drug-appropriate lever in at least 8 of 10 consecutive sessions, after which the animal proceeded to the testing phase [16].

During the testing phase, substitution tests were conducted with ascending doses, as the discriminative stimulus properties of compounds are concentration-dependent, making it impossible to predetermine subjectively equivalent doses. The first group of animals, trained to discriminate RU-1205, received RU-1205 (3.5, 35, or 350 µg, i.c.v.) 10 min before the session. Subsequently, these animals underwent substitution tests with the selective kappa-opioid agonist U-50488 (1, 10, 100 µg, i.c.v.) and the p38 MAPK inhibitor SB203580 (0.01, 0.1, 1 µg, i.c.v.) to test for generalisation to the training drug.

During its testing phase, the third group received naloxone (200 µg, i.c.v.) 15 min prior to the session, followed by SB203580 (0.01, 0,1, 1 µg, i.c.v.) 10 minutes prior. These animals then underwent substitution tests with a combination of RU-1205 and naloxone (3.5, 35, and 350 µg RU-1205 / 200 µg naloxone, i.c.v.). Tests were conducted twice weekly. Between test sessions, animals underwent maintenance training with ACSF / naloxone or the training dose of their respective compound. For each training drug, the mean number of sessions to acquisition (± SD) was calculated, and dose-effect curves were constructed, plotting the percentage of responses on the drug-associated lever. The experimental timeline is depicted in Figure 2.

Statistical data analysis

Statistical analysis was performed using GraphPad Prism 10.1 (Dotmatics, USA). Data were tested for normality using the Shapiro-Wilk test before applying parametric methods. Spectral and coherence analysis data are presented as mean ± standard error of the mean (M ± SEM). Discriminative stimulus data are presented as mean ± standard deviation (M ± SD), expressed as the percentage of responses on the drug-associated lever. An unpaired Student's t-test was used for comparisons between two independent groups in the LFP analysis. Dose-effect curves were analyzed using a one-way repeated measures analysis of variance (ANOVA), followed by Dunnett's post hoc test. A $p < 0.05$ was considered statistically significant.

RESULTS

Analysis of LFP spectral characteristics revealed statistically significant changes following the administration of RU-1205 (350 µg, i.c.v.) compared to vehicle ($p < 0.05$). Specifically, an increase in theta band power and a decrease in gamma band power were observed in frontal cortical recordings (Fig. 3a). In parietal cortical recordings, an increase in theta band power was also detected (Fig. 3b). Concurrently, a decrease in delta band power was observed in occipital cortical recordings (Fig. 3c).

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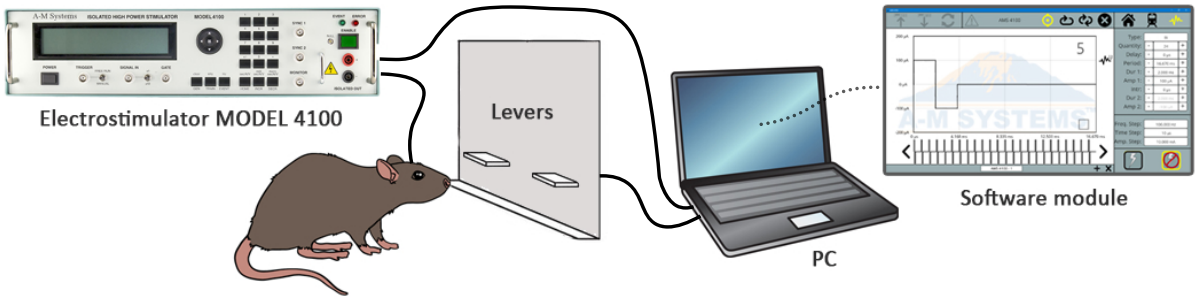

Figure 1 – Operant chamber with two levers, an electrical stimulator, and a personal computer for investigating neuroactive substances in a stimulus discrimination model.

Table 1 – Administration schedule for ACSF (5 µl), naloxone (200 µg), and test compounds during discrimination training.

Group		Week 1	Week 2	Week 3
1	RU-1205 (350 µg i.c.v., *n* = 9)	R-A-R-R-R-A-A	A-R-R-A-R-A-A	A-R-R-R-A-A-R
2	RU-1205 + naloxone (350 µg / 200 µg i.c.v., *n* = 8)	Rn-N-Rn-N-N-Rn-Rn	N-Rn-Rn-N-N-Rn-Rn	Rn-N-N-Rn-Rn-Rn-N
3	SB203580 + naloxone (1 µg / 200 µg i.c.v., *n*=8)	Sn-N-Sn-Sn-N-N-Sn	N-Sn-N-Sn-Sn-N-Sn	Sn-N-Sn-N-Sn-N-N

Note: A — ACSF; N — naloxone; R — RU-1205; Rn — RU-1205+naloxone; Sn — SB203580+naloxone.

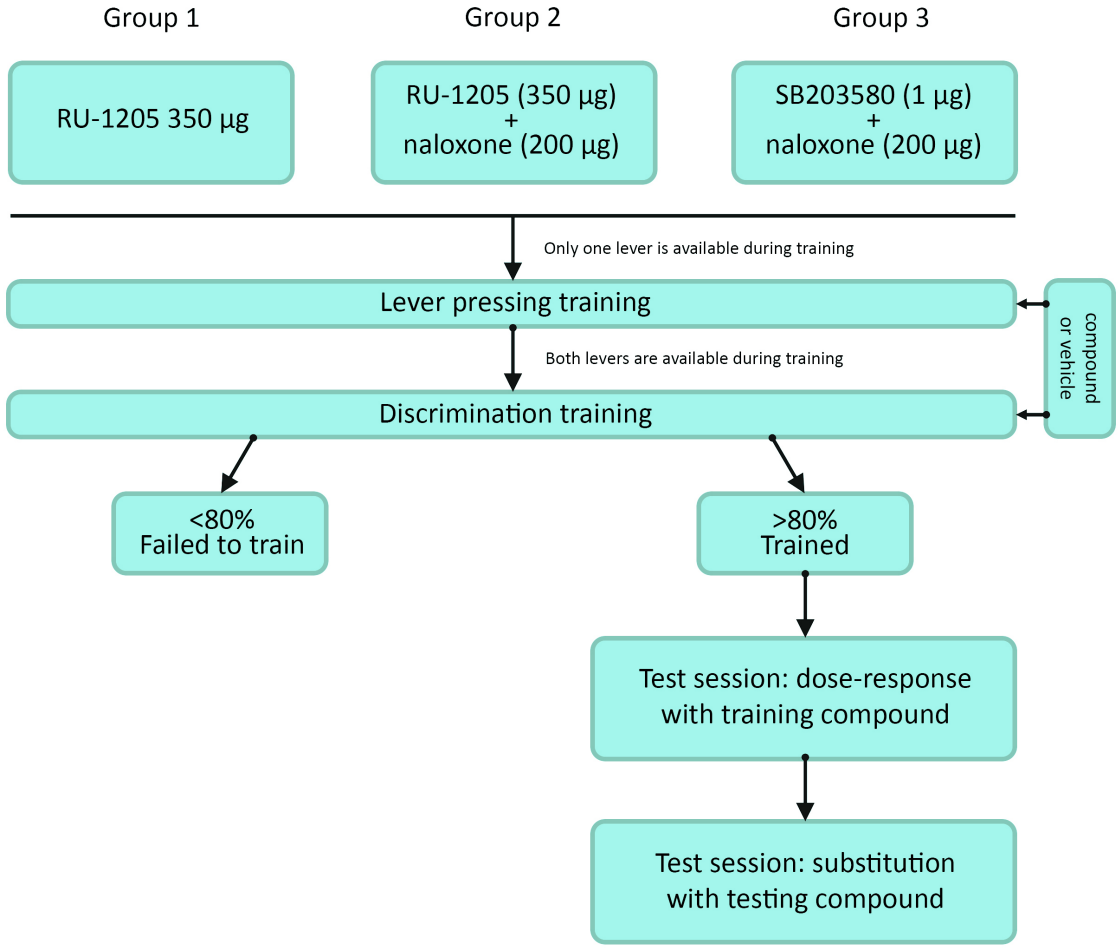

Figure 2 – Schematic of the experimental design for studying the discriminative stimulus properties of the test compounds, encompassing training and testing phases.

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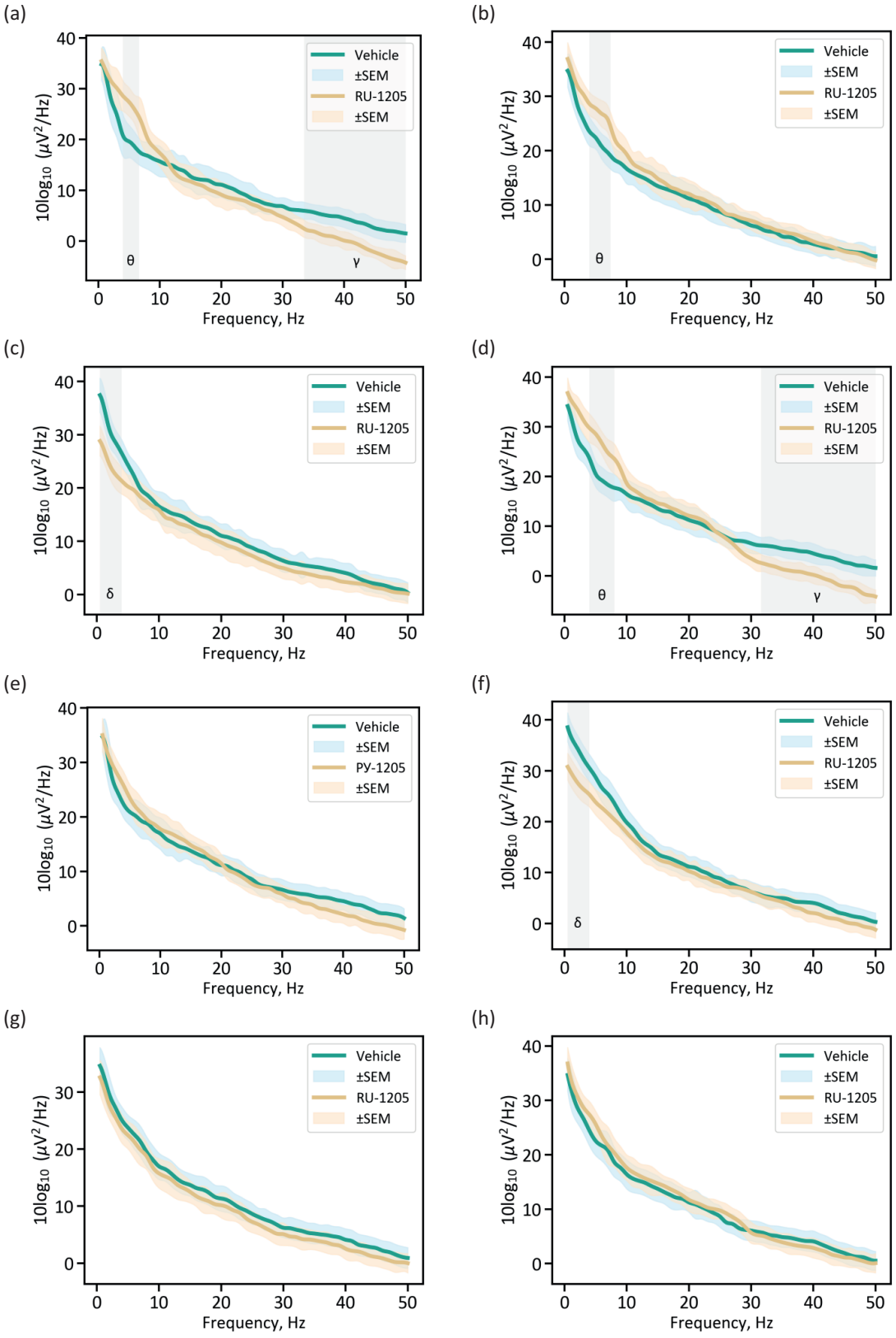

Figure 3 – Spectral power density of LFP oscillations following administration of RU-1205 (350 μg, i.c.v.).
Note: (a) — frontal lead (F); (b) — parietal lead (P); (c) — occipital lead (O); (d) — medial prefrontal cortex (mPFC); (e) — amygdala (Amy); (f) — hippocampus (Hipp); (g) — nucleus accumbens (NAc); (h) — ventral tegmental area (VTA). Each plot displays mean±SEM for spectral power density. Shaded areas indicate frequency bands with statistically significant power deviations relative to vehicle ($p < 0.05$).

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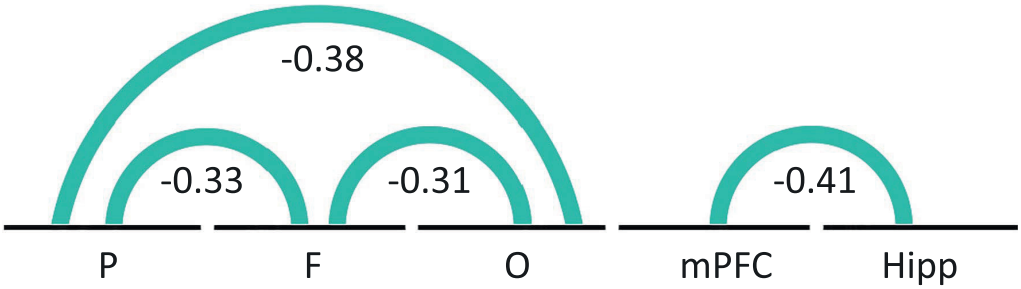

Figure 4 – Effect of RU-1205 on theta band coherence between LFP signals.
Note: the diagram illustrates the connections (coherence values after Fisher's z-transformation) that were statistically significantly altered by RU-1205 (350 µg, i.c.v.) compared to control ($p < 0.05$). F — frontal lead; P — parietal lead; O — occipital lead; mPFC — medial prefrontal cortex; Hipp — hippocampus.

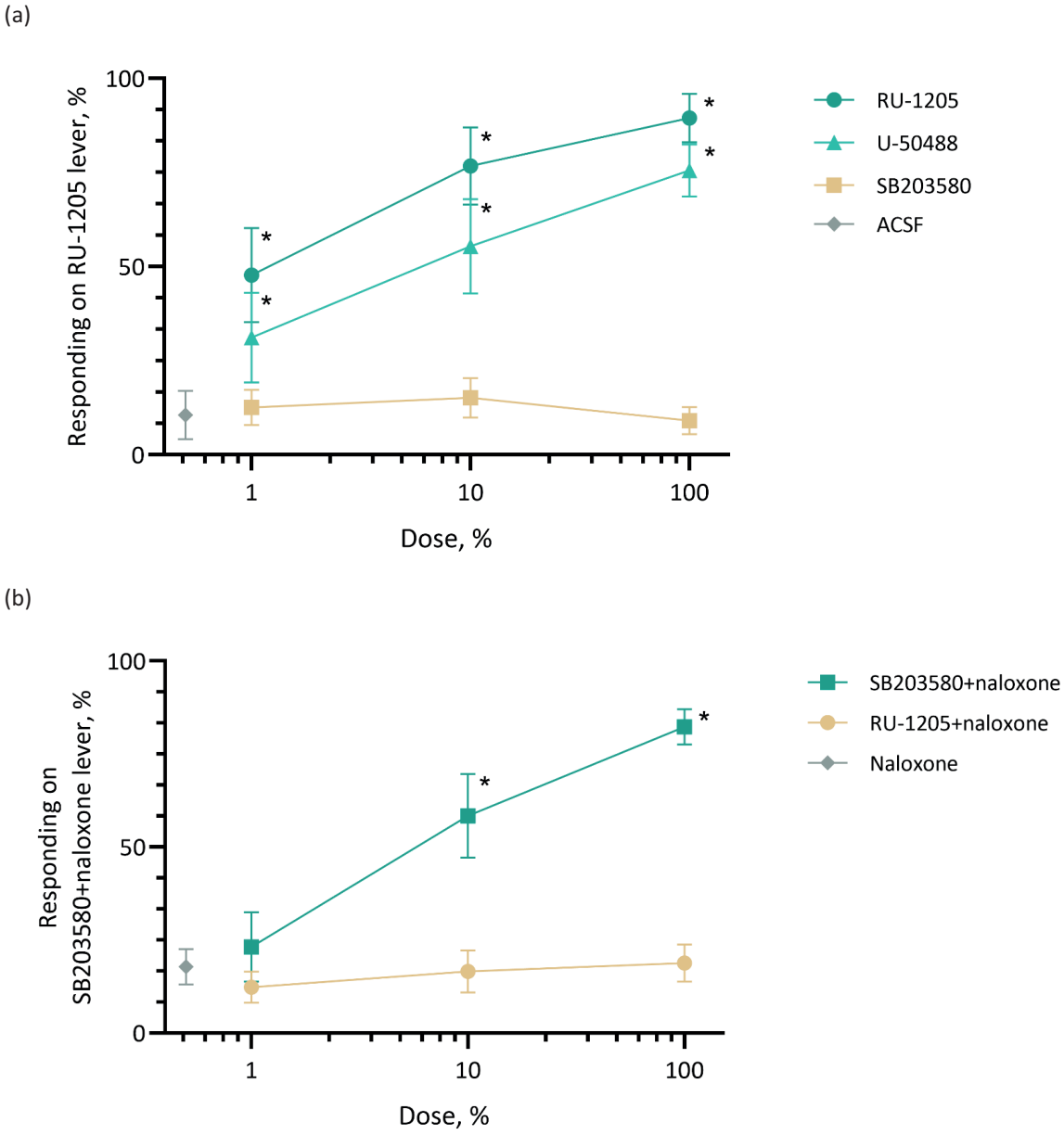

Figure 5 – Effect of test compounds on the percentage of responses on the drug-appropriate lever in the stimulus discrimination paradigm.
Note: (a) — response of rats to RU-1205, U-50488, and SB203580 after training to discriminate RU-1205 (350 µg, i.c.v.); (b) — Response of rats to combinations of SB203580+naloxone and RU-1205 + naloxone after training to discriminate SB203580+naloxone (1 µg / 200 µg, i.c.v.). The highest dose of each test substance was normalized to 100%. Data are presented as mean±SD; $p < 0.05$ compared to control solution.

RU-1205 led to increased theta power and suppressed gamma oscillations in the medial prefrontal cortex (Fig. 3d). A reduction in delta band power was also noted in the hippocampus (Fig. 3f). In contrast, no statistically significant changes were found in the amygdala, nucleus accumbens, or ventral tegmental area.

Next, theta band coherence was assessed across all electrode pairs. RU-1205 significantly decreased coherence for four pairs of leads compared to vehicle ($p < 0.05$), as presented in Fig. 4.

All rats in the first group acquired the discrimination in an average of 18 ± 3.0 sessions. During testing, the training dose of RU-1205 produced 89.44 ± 6.45% responding on the drug-appropriate lever, and RU-1205 dose-dependently increased responding on this lever ($F_{2.060, 16.48} = 208.1$, $p < 0.0001$). U-50488 partially substituted for RU-1205, with rats generalizing to U-50488 up to 75.56 ± 6.95% at the 100 µg dose, 55.34% (± 12.52, SD) at 10 µg, and 31.11 ± 11.89% at 1 µg ($F_{2.221, 17.77} = 201.7$, $p < 0.0001$) (Fig. 5a).

The second group of animals failed to reach the discrimination criterion during the training period.

The third group acquired the discrimination between SB203580 in combination with naloxone and vehicle in 17,5 ± 3,29 days. The discrimination exceeded the 80% criterion ($F_{2.105, 14.74} = 245.7$, $p < 0.0001$), although the effect of the lowest dose (0.01 µg) did not differ significantly from control. In substitution tests, RU-1205 co-administered with naloxone did not significantly alter responding on the lever associated with SB203580, indicating that RU-1205 lacks additional p38 MAPK-inhibitory activity (Fig. 5b).

DISCUSSION

The effects of kappa-opioid agonists on electroencephalographic activity are well-documented in both animal and human studies. This class of compounds induces specific alterations in cortical activity, notably a characteristic increase in power within the 4–8 Hz range (theta activity enhancement) [8]. The pharmaco-EEG profile of the canonical kappa-opioid agonist U-50488 is consistent with a CNS depressant effect [18]. In studies of U-50488's effects on LFP activity, significant changes have been identified in the nucleus accumbens (NAc) and ventral tegmental area (VTA) — key components of the reward system — as well as in the basolateral amygdala (BLA), a region associated with aversive states and depression. The location and nature of these changes align with the spectral correlates of aversive effects. Theta frequency in the prefrontal cortex and limbic areas (amygdala, hippocampus) is closely linked to fear and avoidance behaviors. It has been noted that gamma activity in the amygdala is suppressed during periods of fear [19, 20], while theta power in the mPFC-BLA circuit increases [21]. Stress can enhance theta waves in the dorsal hippocampus (dHPC), BLA, and amygdala, and gamma frequencies in the dHPC, BLA, and the infralimbic (IL) division of the medial prefrontal cortex [22]. Furthermore, studies have revealed abnormal LFP patterns in the NAc of individuals with depression [23]. In animal models of anxiety, an increase in theta band power (4–12 Hz) has been observed in the NAc [24]. Consequently, it is plausible that previously uncharacterized effects of RU-1205 could be revealed through the analysis of its impact on brain bioelectrical activity.

RU-1205 did not induce significant LFP changes in the NAc, VTA [25], or Amy [26], which is consistent with *in vivo* data demonstrating its lack of dysphoric and depressant properties [11, 27]. The deviations in spectral characteristics from the F, P, mPFC, and Hipp leads in the RU-1205 group correspond with those recorded following U-50488 administration. Some of these changes could potentially serve as LFP markers for the analgesic effects of these kappa-opioid agonists. For instance, EEG gamma waves are reportedly associated with nociception, with gamma activity significantly increasing during a painful stimulus [28]. Additionally, data from human EEG studies indicate that chronic pain relief is associated with an increase in theta power over fronto-medial leads [29].

The subsequent phase of this research investigated coherence, a measure of synchrony between two LFP signals. Previous experiments with U-50488 demonstrated pronounced alterations in phase synchronization [18]. A decrease in coherence was observed between cortical electrodes and between the prefrontal cortex, hippocampus, nucleus accumbens, and ventral tegmental area (effects also seen in depression, cognitive impairment, and with opioid analgesic use), while an increase in connectivity with the amygdala was noted (a characteristic sign of aversive action and stress response) [18]. It is well-established that

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the amygdala and hippocampus are involved in pain perception and negative mood [30]. During states of fear, theta oscillations in the basolateral amygdala, hippocampus, and medial prefrontal cortex become synchronized, a phenomenon thought to underlie the response to aversive stimuli [19]. Likhtik et al. also concluded that mPFC-BLA synchronization is a key factor in anxiogenesis [21]. Notably, morphine has been shown to attenuate theta activity and enhance gamma activity in the NAc, while increasing NAc-VTA coherence [31, 32]; opposite effects were observed for U-50488 [18]. Therefore, these changes in synchrony may represent electrophysiological signatures of the euphoric versus dysphoric actions of substances.

RU-1205 did not produce the full spectrum of effects seen with U-50488. Its impact was limited to changes in cortical connectivity and a reduction in coherence between the prefrontal cortex and the hippocampus. These findings could be related to either the analgesic action of RU-1205 or potential cognitive side effects. For example, disruption of hippocampal-prefrontal connectivity has been shown to impair working memory [33], which is consistent with a known side effect of kappa-opioid agonists [34]. Importantly, no electrophysiological signs of aversive action were found for RU-1205. The mechanism underlying the aversive effects of kappa-opioid agonists (e.g., depression, sedation, dysphoria) is linked to intracellular signaling via the β-arrestin pathway and subsequent activation of p38 MAP kinase. The activity profile of RU-1205 might be explained by ligand-biased signaling (i.e., ligand-dependent selectivity for specific intracellular signaling pathways) [35, 36] or by a dual-target mechanism (kappa-opioid receptor activation combined with p38 MAPK blockade). To test the latter hypothesis, we employed the drug discrimination paradigm.

Our results lead to the conclusion that the pharmacological effects of RU-1205 bear little resemblance to those of SB203580, while showing a high degree of similarity to U-50488. The inability of trained animals to discriminate the effects of RU-1205 in the presence of naloxone suggests that the compound does not possess subjectively perceivable off-target activity.

The electrophysiological approach is a valuable tool for understanding how opioid compounds affect receptor signaling and physiological processes such as pain, respiration, and addiction [37]. Gillis et al., in a comprehensive comparative analysis of GPCR ligands, concluded that the pharmacological properties of biased agonists are determined not only by the degree of imbalance in post-receptor cascade activation but also by other factors [38], such as the intrinsic efficacy of the ligands. Birdsong and colleagues also underscore the importance of employing diverse approaches, particularly electrophysiology, in the study of ligand-biased signaling [37]. Our research was limited to observing changes that are not direct readouts of secondary messenger activity. However, this limitation is offset by the versatility of our approach, which allows for the indirect assessment of a wide range of biochemical processes as they manifest in brain function and behavior.

Limitations of the study

This work has several methodological limitations. The experiments were conducted exclusively on adult male rats, which may limit the generalizability of the findings to females. Bioelectrical activity was recorded at a single, fixed 30-minute time point post-injection, precluding analysis of earlier or later effects. The spectral analysis was confined to the standard EEG range (up to 50 Hz), excluding higher frequency components that could be informative for assessing synaptic activity. The study focused on a limited set of cortical and limbic structures, omitting other brain regions involved in opioid signaling. Finally, while the intracerebroventricular route of administration ensures direct brain exposure and controlled experimental conditions, its clinical relevance is limited, a factor that should be considered when interpreting the results.

CONCLUSION

In summary, we have established that, compared to the selective kappa-opioid agonist U-50488, RU-1205 induces less pronounced changes in LFP activity and lacks the electrophysiological patterns associated with β-arrestin pathway activation and aversive effects. The electrophysiological effects of RU-1205 were confined to cortical regions, the hippocampus, and the prefrontal cortex. The absence of behavioral evidence for p38-inhibitory activity, as confirmed in the drug discrimination model, underscores the relevance of investigating ligand-biased signaling as the potential mechanism for its unique profile in future studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Konstantin Yu. Kalitin — statement of key objectives, analysis of scientific and methodical literature, data processing, writing, and editing of the manuscript; Olga Yu. Mukha — data collection, data processing, writing, editing, and formatting of the manuscript; Alexander A. Spasov — critical revision of draft manuscript with valuable intellectual investment, final manuscript approval. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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AUTHORS

Konstantin Yu. Kalitin — Candidate of Sciences (Medicine), Assistant Professor, Assistant Professor of the Department of Pharmacology and Bioinformatics, senior researcher at the Laboratory of Metabotropic Drugs of the Scientific Center for Innovative Drugs, Volgograd State Medical University. ORCID ID: 0000-0002-0079-853X. E-mail: kkonst8@ya.ru

Olga Yu. Mukha — PhD candidate of the Department of Pharmacology and Bioinformatics, junior researcher at the Laboratory of Metabotropic Drugs, Scientific Center for Innovative Drugs, Volgograd State Medical University. ORCID ID: 0000-0002-0429-905X. E-mail: olay.myha14@gmail.com

Alexander A. Spasov — Doctor of Sciences (Medicine), Professor, Head of the Department of Pharmacology and Bioinformatics, Head of the Department of Pharmacology and Bioinformatics of the Scientific Center for Innovative Drugs, Volgograd State Medical University; Academician of the RAS. ORCID ID: 0000-0002-7185-4826. E-mail: aaspasov@volgmed.ru