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Electrophysiological effects of kappa-opioid analgesic, RU-1205, using machine learning methods

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The study is focused to the investigation of a new kappa-opioid agonist RU-1205, which exhibits an analgesic effect without causing dysphoric or aversive actions. It is assumed that this effects may be due to its functional selectivity, or the presence of an additional mechanism of action that involves blocking p38 mitogen-activated protein kinase (MAPK).

The aim of the study was an experimental identification of RU-1205 mechanisms of action associated with the inhibition of MAPK p38 and functional selectivity for kappa opioid receptors.

Materials and methods. The LFP activity was recorded in the male rats weighing 260–280 g (*n*=62) and implanted with chronic cortical and deep electrodes, after the intracerebroventricular administration of the well-studied reference substances: the selective kappa-opioid agonist U-50488 100 μg; the MAPK p38 blocker SB203580 1 μg; and the investigational compound RU-1205 at 350 μg. The weighted phase lag index (WPLI) was calculated. Subsequently, machine learning methods were employed to reduce the dimensionality and extract connectivity features using the principal component analysis method, then a signal classification was performed (models based on Gaussian processes). Using the local patch-clamp technique in the "whole-cell" configuration, the spike activity of pyramidal neurons in the basolateral amygdala was studied. Neurons were identified by their accommodation properties. After local perfusion of the test compounds, 3 dose-response curves were obtained for: (1) U-50488 at concentrations ranging from 0.001 to 10 μM; (2) combinations of U-50488 (0.001–10 μM) and RU-1205 (10 μM); and (3) the combinations of U-50488 (0.01–10 μM) and RU-1205 (100 μM).

Results. The developed models made it possible to classify the compound RU-1205 as a "non-inhibitor" of MAPK p38 with a high probability. The results obtained were confirmed in patch-clamp experiments on acute brain slices where it was demonstrated that U-50488 statistically significantly increases the spike activity of pyramidal neurons of the basolateral amygdala (p <0.05), and RU-1205 interacts with U-50488, competitively suppressing its effect on the spike activity of neurons.

Conclusion. The findings suggest that compound RU-1205 displays properties consistent with a functional kappa agonist activity and does not have a significant effect on MAPK p38. The study demonstrates the possibility of integrating electrophysiological measurements and advanced data analysis methods for a deep understanding of drug action and underscores the potential for further research in this area.

Keywords: kappa-opioid analgesics; electrophysiology; brain connectivity; patch-clamp; machine learning methods; p38 MAPK

Abbreviations: BBB – blood-brain barrier; CNS – central nervous system; ACSF – artificial cerebrospinal fluid; BLA – basolateral amygdala; LFP – local field potential; p38 MAPK – p38 mitogen-activated protein kinase; WPLI – weighted phase lag index.

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Электрофизиологическое исследование каппа-опиоидного анальгетика РУ-1205 с применением методов машинного обучения

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Исследование посвящено изучению нового каппа-опиоидного агониста РУ-1205, который проявляет анальгетическое действие, при этом не вызывает дисфорических или аверсивных эффектов. Предполагается, что это может быть обусловлено функциональной селективностью, либо наличием дополнительного механизма действия, который связан с блокированием p38 митоген-активируемой протеинкиназы (MAPK).

Цель. Экспериментальное выявление механизмов действия соединения РУ-1205, связанных с ингибированием MAPK p38 и функциональной селективностью в отношении каппа-опиоидных рецепторов.

Материалы и методы. Крысам массой 260–280 г (n=62) имплантировали хронические корковые и глубокие электроды, выполняли регистрацию LFP-активности после интрацеребровентрикулярного введения веществ с хорошо изученными фармакологическими свойствами (селективный каппа-опиоидный агонист U-50488 100 мкг и блокатор MAPK p38 SB203580 1 мкг), а также изучаемого соединения РУ-1205 350 мкг. Рассчитывали взвешенный индекс фазовой задержки (WPLI), после чего применяли методы машинного обучения с целью снижения размерности и получения интегративных характеристик коннективности (метод главных компонент), затем выполняли классификацию сигналов (модели на основе гауссовских процессов). С помощью метода локальной фиксации потенциала в конфигурации “whole-cell” исследовали спайковую активность пирамидных нейронов базолатеральной миндалины. Нейроны идентифицировали по их свойствам аккомодации. После локальной перфузии исследуемых соединений были получены 3 кривые доза-эффект для: (1) U-50488 в концентрациях от 0,001 до 10 мкМ; (2) комбинации U-50488 (0,001–10 мкМ) и РУ-1205 (10 мкМ); и (3) комбинации U-50488 (0,01–10 мкМ) и РУ-1205 (100 мкМ).

Результаты. Разработанные модели позволили с высокой вероятностью классифицировать соединение РУ-1205 как «неингибитор» MAPK p38. Полученные результаты находят подтверждение в экспериментах «Patch Clamp» на живых срезах мозга, где было продемонстрировано, что U-50488 статистически значимо увеличивает спайковую активность пирамидных нейронов базолатеральной миндалины (p <0,05), при этом РУ-1205 взаимодействует с U-50488, конкурентно подавляя его действие на спайковую активность нейронов.

Заключение. Таким образом, это позволяет предполагать, что соединение РУ-1205 проявляет функциональную каппа-агонистическую активность и не оказывает значимого влияния на MAPK p38. Исследование демонстрирует возможность интеграции электрофизиологических измерений и передовых методов анализа данных для глубокого понимания нейрональных механизмов фармакологического действия, а также подчеркивает перспективность дальнейших исследований в данном направлении.

Ключевые слова: каппа-опиоидные анальгетики; электрофизиология; коннективность мозга; патч-зажим; методы машинного обучения; p38 MAPK

Список сокращений: ГЭБ – гематоэнцефалический барьер; ЦНС – центральная нервная система; ACSF – искусственная спинномозговая жидкость; BLA – базолатеральная миндалина; LFP – локальный полевой потенциал; p38 MAPK – p38 митоген-активируемая протеинкиназа; WPLI – взвешенный индекс фазовой задержки.

INTRODUCTION

A pain syndrome is one of the most common and challenging medical problems fasing our society [1]. Regardless of its location or nature, as well as its nature (acute or chronic), the use of analgesics remains a primary method for pain control [2].

Most of the narcotic analgesics used in clinical practice act on the mu-opioid receptor type; however, the use of these drugs is associated with serious side effects, such as the development of dependence, nausea, respiratory depression, constipation, and others. [3]. It was found that agonists of kappa-opioid receptors have an analgesic effect without causing adverse reactions in the respiratory and gastrointestinal

tracts, and have also a low abuse potential [4]. However, their application is constrained due to aversive effects such as depression, dysphoria, and hallucinations [5].

Kappa-opioid receptors are localized in the central and peripheral nervous systems. They are involved in the regulation of pain, mood, behavior, a motor activity, and perform neuroprotective and neuroendocrine functions [5, 6]. Active conformations of the receptor provide the recruitment of effector molecules, the main of which are G-proteins and beta-arrestins. It is assumed that the analgesic effect of kappa-opioid agonists is associated with activation of G-proteins and a subsequent inhibition of adenylate cyclase, increasing potassium and decreasing calcium kinds of conductivity [7]. The activation of the beta-arrestin pathway leads to suppression of G-protein signaling, desensitization and internalization of the kappa-opioid receptor, which is one of the reasons for the development of tolerance. In addition, beta-arrestins can initiate protein kinase p38, which is an important component of the mechanism for the development of aversive side effects [8].

Currently, an urgent task is to search for safer kappa-opioid receptor agonists with an analgesic activity [9]. Among the solutions to this problem, the following areas can be identified: 1) the creation of drugs that have only a peripheral effect, i.e, they do not penetrate the blood-brain barrier (BBB) and do not act on the central nervous system (CNS); 2) the search for functionally selective substances that activate a specific signaling pathway, e.g., G-proteins without involving beta-arrestins [10]. It is assumed that the conformational changes of the receptor upon binding to the agonist determine its functional selectivity.

Our research focuses on the new kappa-opioid agonist RU-1205. Unlike classical representatives of this class, the compound RU-1205 exhibits pronounced analgesic, anticonvulsant [11, 12] and neuroprotective [13] actions while not causing dysphoric or aversive effects. This property may be due to the functional selectivity, or the presence of an additional mechanism of action that is associated with blocking p38 mitogen-activated protein kinase (MAPK) p38, since it was previously shown that the p38 inhibitor SB203580 can completely eliminate the aversive effect of kappa-opioid agonists [14].

Since aversive effects can manifest at both the cellular and systemic levels of the neuronal organization, the patch clamp method and recorded local field potentials (LFPs), followed by the calculation of the weighted phase lag index (WPLI) and the application of machine learning (ML) as a tool for identifying the differences and similarities among ligands with well-studied pharmacological properties, such as U-50488, and the p38 MAPK inhibitor SB203580, were used.

THE AIM of the study was an experimental identification of RU-1205 mechanisms of action associated with the inhibition of MAPK p38 and functional selectivity for kappa opioid receptors.

MATERIALS AND METHODS

Study design

The study was conducted in two main stages. In the first stage, the local field potential (LFP) was recorded after the administration of the test substances, then the weighted phase lag index (WPLI) was calculated, and the resulting data were analyzed using ML methods.

At the second stage, the interaction of the compound RU-1205 with U-50488 at the cellular level was studied using the patch clamp method in order to clarify the mechanism of RU-1205 action and further validate the results of the LFP classification.

Test compounds

The compounds used in this study were as follows: 9-(2-morpholinoethyl)-2-(4-fluorophenyl) imidazo[1,2-a]benzimidazole – RU-1205 (synthesized at the Research Institute of Physical and Organic Chemistry of Southern Federal University, Russia); compound U-50488 (Sigma Aldrich, USA); compound SB203580 (Sigma Aldrich, USA).

Study duration and location

The study was conducted in the period from May to September 2023. All experimental procedures were performed in the electrophysiological research laboratory of the Scientific Center for Innovative Drugs with Pilot Production of Volgograd State Medical University (Russia).

Ethical approval

Animal experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, the principles of Good Laboratory Practice (GLP) (GOST 33044-2014, 2021), as well as the ARRIVE (Animal Research: Reporting of In Vivo Experiments). The study was approved by the Local Ethics Committee of Volgograd State Medical University (Registration No. IRB00005839 IORG0004900, Minutes No. 2022/096 dated Jan 21, 2022).

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Animals

Sixty-two white male rats, weighing 260–280 g (*n*=62), were used in these studies. Animals were subjected to a 12-hour light/dark cycle, the ambient temperature was 22±2°C, with food and water available *ad libitum*. The rats had been bred in the vivarium of the Scientific Center for Innovative Drugs with Pilot Production at Volgograd State Medical University (Russia).

Surgical interventions

Implantation of stainless-steel electrodes (⌀ 0.1 mm) was carried out under isoflurane anesthesia (Laboratories Karizoo, S.A., Spain) using an available rodent inhalation anesthesia system (Ugo Basile Veterinary anesthesia workstation 21100, Italy). The electrodes were stereotaxically inserted through burr holes to target sites in accordance with the coordinates relative to the bregma.

Cortical electrodes: F – anteroposterior axis (AP)=0.00; mediolateral axis (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 the intracerebroventricular (i.c.v.) administration of drugs, a 21-gauge stainless steel guide cannula was implanted into the right lateral ventricle. The cannula was positioned based on the stereotaxic coordinates relative to the bregma: AP=-0.6 mm, ML=1.6 mm, DV=4.0 mm. The intracerebroventricular route of administration had been chosen to enhance the bioavailability of the substances and to prevent the effects outside the CNS, which could potentially mediate the secondary impacts on the brain functions and distort the experimental results. Electrodes and cannula were fixed on the skull with self-hardening plastic (Protacryl-M, Ukraine) and two stainless steel screws. After the surgery, the animals were kept in individual cages. For two days, the animals were treated with antibiotic prophylaxis with ciprofloxacin 50 mg/kg w/w. The postoperative period lasted 5–7 days.

LFP signal recording and administration of test substances

After adaptation, the animals (*n*=30) were injected with ACSF 5 µl (a control sample), 350 µg of compound RU-1205, 100 µg of compound U-50488, 1 µg of compound SB203580, and a combination of SB203580 and U-50488 (1 µg and 100 µg, respectively) intracerebroventricularly. Intervals of 7 days were maintained between the administrations of substances to achieve a complete excretion (at least 97% based on the elimination half-life).

Doses for compounds U-50488 [15] and SB203580 [16] were selected based on literature data. Compound RU-1205 was administered at a dose equivalent to the ED_{80} in pain models. 30 minutes after the administration of the compounds, LFP recordings were obtained for 10 minutes. LFP was recorded in a monopolar montage with a common average reference using a laboratory electroencephalograph (ISS, Russia). The signals were digitized at a sampling rate of 500 Hz.

WPLI analysis

To assess the functional connectivity between electrode pairs, a weighted phase lag index was used (Fig. 1). The WPLI calculation between the electrodes was carried out using the MNE Python Software package v.1.6.1[1] (BSD-3-Clause license) for the following frequency ranges: delta 0.5–4 Hz, theta 4–8 Hz, alpha 8–12 Hz, beta 12–30 Hz and gamma 30–50 Hz. The obtained data were analyzed by the principal component analysis method using the Graphpad Prism 10.1 (Dotmatics, USA) with an academic license.

Classifier ML-model construction

To build models based on ML algorithms, the open-source Python library scikit-learn 1.3.2[2] (BSD 3-Clause License) was used. Two classification models were established based on Gaussian processes: GPC-BO-v.02.10-5.2308 (GaussianProcessClassifier with parameters 10.0 * RBF(5.0), optimizer=None) and GPC-BO-v.02.10-10.2308 (GaussianProcessClassifier with parameters 10.0 * RBF(10.0), optimizer=None). After training the models, confusion matrices constructed and a 5-fold cross-validation was performed.

Local fixation of cell potential in “whole cell” configuration

Coronal brain slices, 500 µm thick and containing the BLA, were made at the level of the basolateral complex 2.5–3.5 mm caudal to bregma using a vibratome (Campden 7000smz-2, UK). Each slice was transferred to

[1] Zenodo. MNE-Python (v1.6.1). Available from: https://zenodo.org/records/10519948
[2] Scikit-learn. Machine Learning in Python. - [Electronic resource]. – Access mode: https://scikit-learn.org/stable/index.html

a recording chamber, filled with artificial cerebrospinal fluid (ACSF), (117 mM sodium chloride, 4.7 mM potassium chloride, 1.2 mM sodium dihydrophosphate, 2.5 mM calcium chloride, 1.2 mM magnesium chloride, 25 mM sodium hydroarbonate, and 11 mM glucose, aerated with a mixture of 95% oxygen and 5% carbon dioxide at pH=7.4) at 31±1°C at a flow rate of 2 mL/min.

The spike activity of basolateral amygdala (BLA) neurons was recorded using a ×40 water-immersion microscope objective (Olympus BX51, Japan) using the standard method of whole-cell potential fixation ("Patch Clamp" in "whole-cell" configuration). Pyramidal cells in the BLA were identified based on their accommodation properties in response to a sustained depolarizing intracellular current injection (200 pA, 500 ms). The recording pipettes made from borosilicate glass were filled with a solution containing: 122 mM K-gluconate, 5 mM sodium chloride, 0.3 mM calcium chloride, 2 mM magnesium chloride, 1 mM ethylene glycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 10 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), 5 mM Na_2-ATP, and 0.4 mM adenosine 5′-triphosphate disodium salt (Na_2-GTP), pH adjusted to 7.2–7.3 with potassium hydroxide (osmolarity adjusted to 280 mOsm/kg with sucrose). The recordings were amplified by a HEKA Patch Clamp EPC10 USB (HEKA Elektronik, USA), analyzed by PatchMaster (HEKA Elektronik, USA) software, Asus microXperts PC (ASUS, Taiwan), PatchMaster software (HEKA Electronik, USA).

Three dose-response curves evaluating the effects of U-50488, a kappa opioid receptor agonist, on the spike activity of pyramidal neurons both in the absence and presence of RU-1205, were obtained. The three experimental conditions included: (1) treatment with U-50488, applied by local perfusion at concentrations ranging from 0.001 to 10 µM (*n*=8); (2) treatment with a combination of U-50488 (0.001–10 µM) and RU-1205 (10 µM) (*n*=8); and (3) treatment with a combination of U-50488 (0.01–10 µM) and RU-1205 (100 µM) (*n*=8). Only one neuron was recorded in each brain slice following the administration of increasing doses of compounds. ACSF served as a vehicle control in all experiments.

Statistical processing

Logistic regression analysis was used to model dose-response curves. Subsequently, the key statistical metrics and coefficients were calculated: the maximum effect (E_{max}), the coefficient of determination (R^2) as a statistical measure of model quality, and the slope coefficient which represents the change in the log odds for each unit increase (or decrease) in the predictor variable by 1.0. After verifying the normal distribution with the Shapiro–Wilk test, the groups were compared by analysis of variance (ANOVA) with repeated measures followed by Dunnett's test using GraphPad Prism 10.1. The obtained data are presented as Means±SEM. A significance level of p <0.05 was considered significant.

RESULTS AND DISCUSSION

WPLI values were calculated for 64 electrode pairs and 5 frequency bands (140 parameters for each signal). Taking into account the multicollinearity among the features, a further analysis was performed by the principal component analysis in order to reduce the number of variables by combining them into integrative connectivity constructs based on the structure of relationships between them. The first 2 most significant components were identified, which explain 58.32% of the variability (eigenvalues >1).

It has previously been established that the aversive effect of kappa opioid agonists is closely related to the amygdala [17]. Local microinjection of SB203580 (p38 MAPK inhibitor) into the amygdala eliminated the aversive effect of the compound U-50488 (p38 MAPK activator) [18]. Our results showed an increased connectivity in amygdala with various brain regions upon the U-50488 administration (ΔWPLI >0.3 compared to control). Furthermore, these changes in amygdala connectivity were not observed when combining SB203580 with U-50488, thereby confirming an antagonistic interaction between these drugs. Considering that the amygdala-related variables had high loading coefficients (>0.7) within PC1, it can be assumed that the features related to the effect of drugs on p38 MAPK were reflected in this principal component.

The next step was to construct classifier models (Fig. 2) to assess the probability of the compound RU-1205 belonging to the 'inhibitor' and 'non-inhibitor' classes. To improve the stability, predictive reliability, and robustness, a method with a kernel function (Gaussian Process Classifier) was chosen.

According to the forecast estimation, compound RU-1205 does not exhibit a MAPK p38 inhibitory activity in the central nervous system, as determined with an average probability of at least 89.44%. To confirm the validity of the models, confusion matrices and the results of a 5-fold cross-validation are presented (Fig. 3, 4).

The second stage of the work is based on the study where it was found that blocking kappa opioid receptors in the amygdala leads to a decrease in the spike activity of pyramidal neurons [19].

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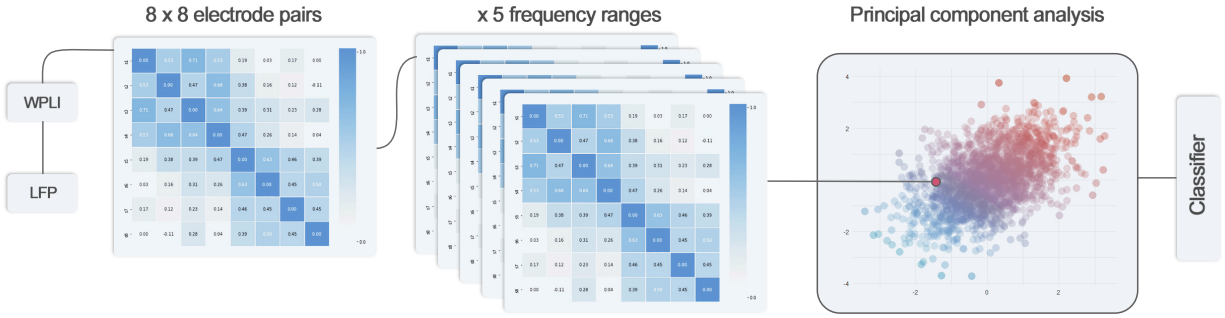

Figure 1 – Schematic diagram of mathematical analysis sequence of electrophysiological data
Note: After calculating the weighted phase latency index (WPLI), reflecting connectivity between brain regions, including the cortex, hippocampus, amygdala, ventral tegmental area, prelimbic cortex, and nucleus accumbens (8×8 electrodes ×5 rhythms). The data were processed by the principal component analysis and then fed to the inputs of a classifier ("Gaussian Process Classifier", scikit-learn).

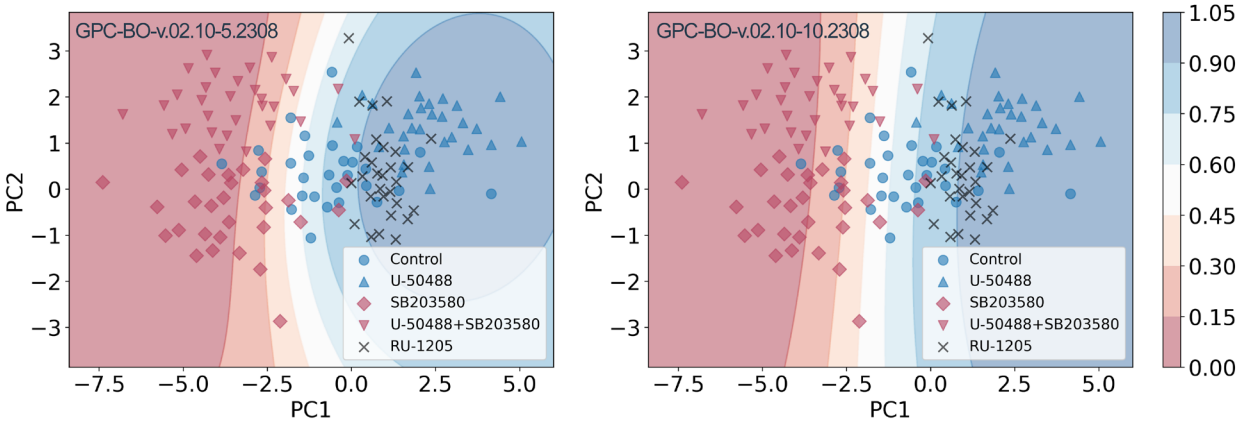

Figure 2 – Decision maps based on "Gaussian Process Classifier" (scikit-learn)
Note: "Inhibitors" of MAPK p38, including the subsets "SB203580" and "SB203580+U-50488", are indicated in red; "non-inhibitors" of MAPK p38 comprising the 'control' subset and the "U-50488" subset, are indicated in blue. Predicted coordinates for the RU-1205 signal subset were established. The average probabilities of RU-1205 signals belonging to the "non-inhibitor" class, are as follows: 0.922584 and 0.894469 as determined by models "GPC-BO-v.02.10-5.2308" (left) and "GPC-BO-v.02.10-10.2308" (right), respectively.

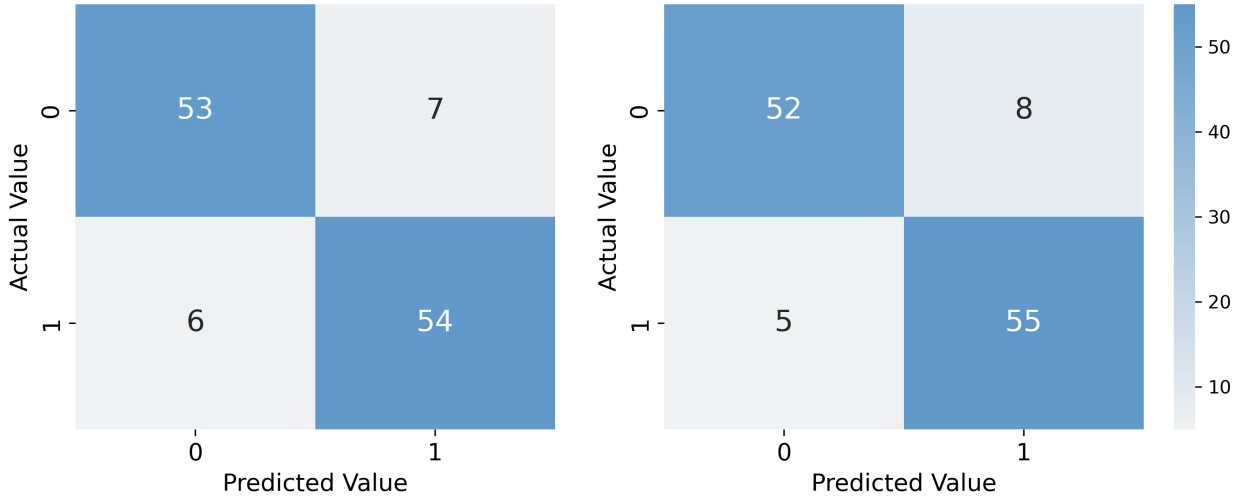

Figure 3 – Confusion matrices for models "GPC-BO-v.02.10-5.2308" (left) and "GPC-BO-v.02.10-10.2308" (right)
Note: class 0 – "inhibitors" of p38 MAPK; class 1 – "non-inhibitors" of p38 MAPK.

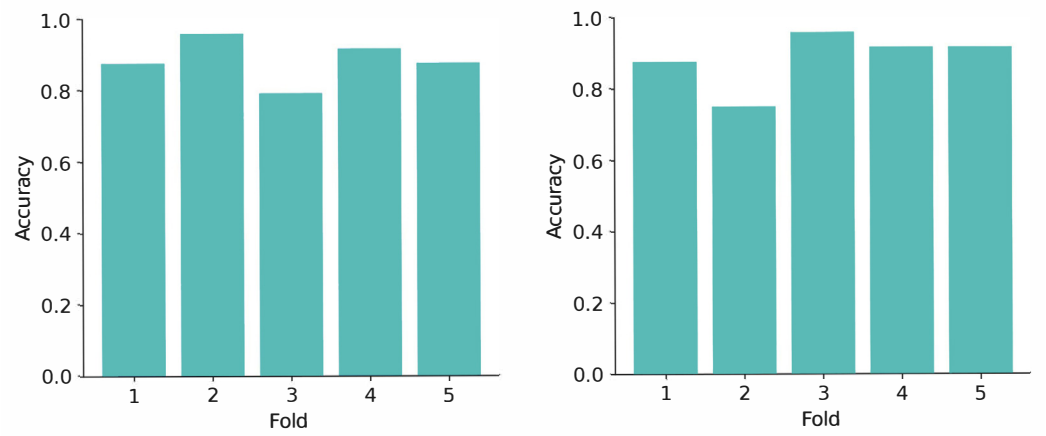

Figure 4 – Results of 5-fold cross-validation of models "GPC-BO-v.02.10-5.2308" (left) and "GPC-BO-v.02.10-10.2308" (right)

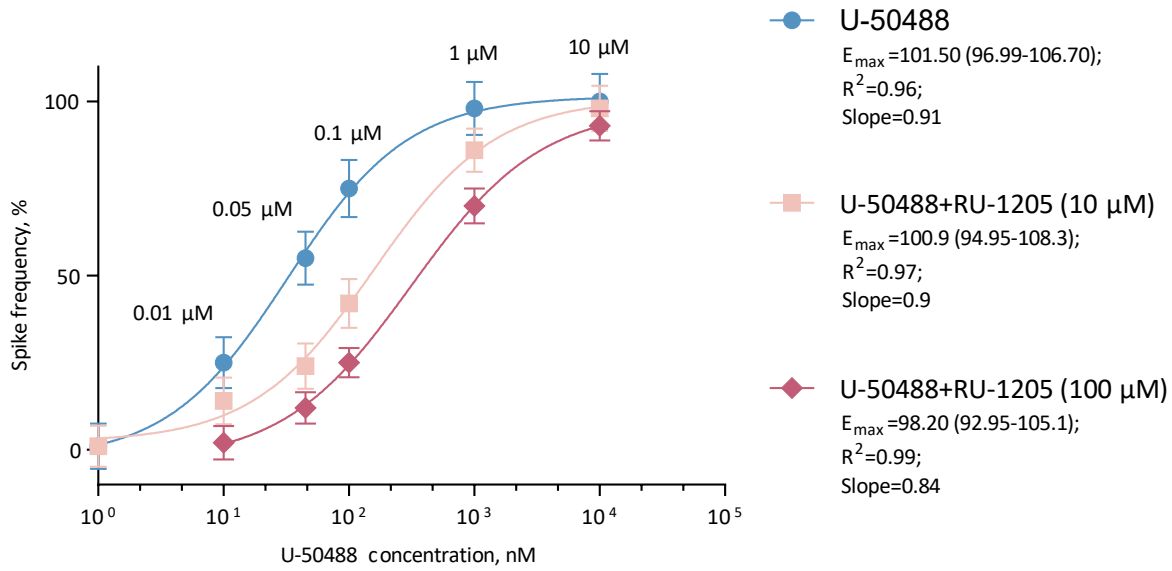

Figure 5 – Dose-dependent effects of compound U-50488 (concentrations shown in the graph) on the spiking activity of amygdala pyramidal neurons in combination with compound RU-1205 (concentration of compound RU-1205 – 0, 10, 100 µM)

A similar effect was observed when blocking CRF1 receptors, which, like kappa-opioid receptors, exert their effects through the beta-arrestin pathway and the activation of MAPK p38 [20]. It has been also shown that the aversive effect of U-50488 was completely eliminated when the substance SB203580 was injected into the amygdala [18]. To determine the effect of the compound RU-1205 (at a concentration of 10 µM and 100 µM) on U-50488-induced spiking activity of pyramidal neurons in the basolateral amygdala, RU-1205 was applied in combination with increasing concentrations of U-50488.

U-50488 was found to dose-dependently increase the firing rate of BLA pyramidal neurons starting at a concentration of 0.01 µM (p <0.05). The obtained dose-dependent curves (Fig. 5) made it possible to conclude that the combination of U-50488 and RU-1205 results in the competitive antagonism. This is evidenced by a rightward shift in the response curves without a significant change in the slope or the maximum effect value (plateau).

It has been shown in various studies that kappa opioid agonists can affect the brain's bioelectrical activity [21]. The effect of opioid drugs is most pronounced in such brain structures as the cortex [22], hippocampus [23], mesolimbic system [24, 25] and amygdala [26]; therefore, deep electrodes were implanted in these areas. It was previously found that a p38 MAPK inhibitor alters neuronal activity in the hippocampus and also reduces EEG power in the

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gamma frequency range in mice [27]. Based on this, it was hypothesized that analyzing the effects of kappa opioid agonists and p38 blockers on LFP could provide an insight into the mechanism of action of the benzimidazole derivative RU-1205 with a similar activity profile. Given the nonspecific nature of spectral changes in LFP, an attempt to extract signs of a pharmacological action from a set of WPLI functional connectivity matrices was made. WPLI was chosen as a method for quantifying connectivity due to its higher resistance to the volume conduction and a lower sensitivity to noise [28]. WPLI is widely used in electroencephalographic (EEG) research [29], electrocorticographic (ECoG) studies [30], and LFP analysis, including studying the effects of substances with a psychotropic activity [31].

To further analyze the resulting WPLI scores after administration of p38 MAPK inhibitors and kappa opioid agonists, a combined approach was adopted, including the principal component analysis and construction of a Gaussian process regression model for a signal classification. Principal component analysis is used in signal processing and ML to reduce the dimensionality of the data while preserving as much information as possible. The performance of the method, when combined with various classifier models, has been confirmed in the EEG studies [32]. The Gaussian process regression model has also shown a high efficiency (94% accuracy achieved for three classes) in analyzing the features of EEG signals associated with stress levels [33], which seems important since kappa opioid agonists are distinguished by their ability to provoke stress and anxiety.

The advantage of the proposed approach is that a nervous tissue is an ideal detector of a neuro- or psychotropic substance, since its effects are specifically reflected in bioelectrical activity, the decoding of which makes it possible to construct highly accurate predictions. Modern analytical methods make it possible to identify more subtle and complex signal patterns associated with a pharmacological action. This is evidenced by the consistent growth in scientific interest and the rising number of studies focusing on the automated EEG classification [34, 35]. One significant area of focus involves classifying EEG, ECoG, and LFP signals to discern the psychotropic effects and elucidate the mechanisms of action of the test substances [36, 37].

To verify the reliability of the obtained conclusions regarding the mechanism of the RU-1205 action, additional studies were carried out using the method of local potential fixation on live rat brain slices. The presented results allow the authors to conclude that the compound RU-1205 does not exhibit an activity similar to SB203580, but competes for a common binding site with the compound U-50488, thus eliminating its aversive effect, an indirect sign (correlate) of which, within this model, is an increased spike activity of neurons in the basolateral complex of the amygdala. Such a phenomenon is a characteristic sign of functional selectivity; in particular, the same effect was found for the functional agonist 6′-GNTI, which attenuates the arrestin-mediated effects of unbiased agonists [38]. At the same time, there were no signs of p38-inhibitory activity [39], when combining the compound U-50488 and SB203580, the phenomena of noncompetitive antagonism expressed as a change in the angular coefficient and a reduction in the magnitude of the maximal effect of U-50488 (p <0.05), were recorded.

Study limitations

It should be noted that the approach used to classify LFP has a number of limitations. A primary concern is the problem of volume conductivity. Electrical signals generated by neurons can propagate in brain tissue, leading to distortion and mixing of signals from different sources. It should be also taken into account that in many brain structures most of the LFP activity originates from remote current sources rather than ones local to the electrode [40]. Inter- and intra-individual variability, noise, nonlinearity, complexity and other characteristics of neuronal signals limit the accuracy of the pharmaco-electroencephalographic technology.

In addition, there is a risk of high uncertainty in case of Out-Of-Distribution ("Out-Of-Distribution"). Therefore, another sample of signals obtained after the administration of the combination “SB203580+U-50488”, which simulates a dual mechanism of action, was also included in the training data set to mimic the dual mechanism of action, but not to completely eliminate this problem.

Conducting a study with repeated measurements, where different drugs are tested on the same group of animals, has several disadvantages, including carryover effects, in which the effect of one drug persists and influences subsequent responses, and order effects, where the sequence of the drug administration significantly affects the results. Training for an effect can alter the response of a laboratory animal over time, potentially skewing the results. This study design may have a limited generalizability due to the homogeneous pool of subjects when extrapolating results to the general population, but, on the other hand, it eliminates the contribution of interclass variability and allows for better performance of the classifier. In addition, when

substances are administered sequentially, the need to wait for their complete (or near-complete) elimination not only prolongs the study's duration but also heightens the risk of the animal being excluded from the experiment due to the development of complications or death.

CONCLUSION

The study presents a comprehensive approach to analyze a brain connectivity, which includes recording electrophysiological data and using machine learning methods to classify pharmacological compounds based on LFP changes. The integrative connectivity characteristics derived from WPLI calculations and principal component analysis, has been identified. These characteristics were associated with the effects of kappa opioid agonists and p38 MAPK inhibitors on different brain parts. The Gaussian process classifier made it possible to classify the compound RU-1205 as a "non-inhibitor" of p38 MAPK with a probability of 0.89.

The results obtained were confirmed in patch clamp experiments on live brain slices. It was demonstrated that RU-1205 interacts with U-50488, competitively suppressing its effect on the spike activity of BLA pyramidal neurons. In contrast, the p38 MAPK inhibitor SB203580 inhibits the spike activity induced by U-50488 non-competitively. This suggests that compound RU-1205 exhibits a functional kappa agonist activity and does not have a significant effect on p38 MAPK.

The study illustrates the potential for combining electrophysiological measurements with the advanced data analysis methods to gain a comprehensive understanding of the neuronal mechanisms of a pharmacological action, and also highlights the promise of further research in this direction.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct the study and preparation of the article, read and approved of the final version before the publication). 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 the draft manuscript with valuable intellectual investment, a final manuscript approval.

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