Pharmacoencephalographic Assessment of Antiphyschotic Agents' Effect Dose-Dependency in Rats

Article *in* Journal of Evolutionary Biochemistry and Physiology · January 2024

DOI: 10.1134/S0022093023060200

9 authors, including:

Yuriy Igorevich Sysoev
St. Petersburg State Chemical Pharmaceutical University
55 PUBLICATIONS **113** CITATIONS
SEE PROFILE

Daria D. Shitc
8 PUBLICATIONS **6** CITATIONS
SEE PROFILE

Maria Mikhailovna Puchik
St. Petersburg State Chemical Pharmaceutical University
9 PUBLICATIONS **6** CITATIONS
SEE PROFILE

Veronika A. Prikhodko
St. Petersburg State Chemical Pharmaceutical University
76 PUBLICATIONS **98** CITATIONS
SEE PROFILE

ISSN 0022-0930, Journal of Evolutionary Biochemistry and Physiology, 2023, Vol. 59, No. 6, pp. 2153–2167. © Pleiades Publishing, Ltd., 2023.
Russian Text © The Author(s), 2023, published in Rossiiskii Fiziologicheskii Zhurnal imeni I.M. Sechenova, 2023, Vol. 109, No. 11, pp. 1665–1683.

EXPERIMENTAL PAPERS

Pharmacoencephalographic Assessment of Antiphyschotic Agents' Effect Dose-Dependency in Rats

Yu. I. Sysoev[a,b,c,d,e,*], D. D. Shits[a], M. M. Puchik[a], I. S. Knyazeva[d], M. S. Korelov[d], V. A. Prikhodko[a,d], I. A. Titovich[a], N. O. Selizarova[a], and S. V. Okovityi[a,d]

[a] *St. Petersburg State Chemical and Pharmaceutical University, St. Petersburg, Russia*

[b] *Pavlov Institute of Physiology of the Russian Academy of Sciences, St. Petersburg, Russia*

[c] *Institute of Translational Biomedicine, Saint Petersburg State University, St. Petersburg, Russia*

[d] *Bechtereva Institute of the Human Brain, St. Petersburg, Russia*

[e] *Sirius University of Science and Technology, Sochi, Russia*
**e-mail: susoyev92@mail.ru*

Received July 1, 2023; revised July 31, 2023; accepted October 2, 2023

Abstract—Pharmacoencephalography (pharmaco-EEG) is a prominent instrument for the pharmacological screening of new psychoactive molecules. This experimental approach has not remained a vestige of neurobiological studies, and can be used successfully to complete today's research objectives. The development and rise to universal use of machine learning techniques opens up novel prospects for the use of pharmaco-EEG data to solve the problems of classification and prognosis. We have previously shown that naïve Bayes classifier (NBC) combined with principal component analysis (PCA) can be used to differentiate between antipsychotic and sedative drug effects as well as to distinguish among the antipsychotics' effects. In the present study, we evaluated the possibility to employ this method to assess the dose-dependency of antipsychotic effects. The experiments were carried out in white outbred male rats with chronically implanted electrocorticographic electrodes. As the agents of interest, we chose two drugs with antipsychotic activity, chlorpromazine and promethazine, in three doses each (0.1, 1, 10 mg/kg and 0.5, 5 and 20 mg/kg, respectively). The training set, used as a reference to determine the pharmacological effects of the agents of interest, included the D_2-dopamine receptor blocker haloperidol, M-cholinergic receptor blocker tropicamide, H_1-histamine receptor blocker chloropyramine, the sedative dexmedetomidine, and the anxiolytic phenazepam. We have shown that the lowest chlorpromazine dose (0.1 mg/kg) can be characterized as antipsychotic with a marked histaminolytic effect, while the highest one (10 mg/kg) exhibits predominantly antipsychotic activity with a cataleptogenic effect. All three doses demonstrated anticholinergic activity, which increased with the dose. For promethazine, we observed a clear dose-dependent shift from antipsychotic action to cataleptogenic, alongside a notable antimuscarinic effect of all doses. None of promethazine doses showed any resemblance to chloropyramine, which probably indicates its anti-dopaminergic and antimuscarinic effects being able to mask its H_1-antihistamine effect in the used dose range. In summary, our results demonstrate that NBC combined with PCA can be used to determine the dose-dependency of antipsychotic agents' effects based on their impact on electrocorticogram parameters. Further development of this method as well as

expansion of psychotropic agent electropharmacogram library would allow for more precise prediction of pharmacological activity of the agents of interest.

DOI: 10.1134/S0022093023060200

Keywords: naïve Bayes classifier, machine learning, pharmacoelectroencephalography, electrocorticography, antipsychotics, chlorpromazine, promethazine

INTRODUCTION

The idea of pharmacoencephalography (pharmaco-EEG) as an experimental research method is based on the ability of pharmacological agents affecting the central nervous system to cause specific changes in the parameters of bioelectrical activity of the brain. In clinical practice, this method is used mainly to assess the effectiveness of pharmacotherapy conducted in patients, for example, in epilepsy [1] or schizophrenia [2]. In studies in small laboratory animals, pharmaco-EEG can be used as a tool of pharmacological screening for agents with previously unknown activity [3] or in-depth study of the effects of known psychotropic drugs [4, 5].

Previously, a novel experimental approach was proposed to identify specific activity and compare the pharmacological profile of antipsychotic agents using the naïve Bayes classifier (NBC), a machine learning algorithm quite widely used in biological and medical research. In this study, a library of rat electrocorticograms (ECoG) recorded after administration of antipsychotic drugs (chlorpromazine, haloperidol, droperidol, tiapride and sulpiride) was collected and used as a training set. For each recording, the ratios of the values of the amplitude-spectral characteristics of the ECoG after drug injection to the corresponding baseline values before injection were calculated. The application of NBC allowed to reveal the specific effect of antipsychotic drugs on the parameters of bioelectrical activity of the rat cerebral cortex, differentiating them from physiological solution (control), as well as the anxiolytic with sedative action phenazepam. Moreover, similarity of the effects of antipsychotics close in chemical structure was noted: butyrophenone derivatives—haloperidol and droperidol, as well as substituted benzamides—tiapride and sulpiride [6].

A characteristic feature of most psychotropic drugs is their broad spectrum of receptor action. For example, antipsychotic agents—phenothiazine derivatives have a pronounced antimuscarinic and H_1-antihistamine effect, which may cause their sedative effect and a number of other central and peripheral adverse reactions. Each of the effects of drugs of this group has its own dose range and, undoubtedly, unique features of influence on ECoG in rats. For further development of the previously proposed approach as a tool for pharmacological screening, it is necessary to supplement the training set with records of the effects of known drugs at different doses.

The aim of this work was to validate a previously proposed method to assess the dose-dependent effects of two antipsychotic drugs, chlorpromazine and promethazine. Particular attention was focused on the possibility of detecting antipsychotic, cataleptogenic, antimuscarinic, H_1-antihistamine, sedative or hypnotic effects of the two drugs studied at three different doses.

MATERIALS AND METHODS

The study was performed on male Wistar stock rats aged 3 months and weighing 250–300 g, obtained from "Kurchatov Institute"—Rappolovo laboratory animal nursery (Leningrad region, Russia). Rats were kept 5 individuals per cage at room temperature 20–22°C and light regime 12 h light 750 lx/12 h darkness. All animals received standard chow (dry full-fed granular extruded compound feed recipe PK-120, LLC "LABORATORKORM", Russia) and had ad libitum access to food and water. Before the experiments, all rats were quarantined for 14 days.

ECoG electrodes were made of nichrome wire 0.5 mm in diameter (for recording and reference electrodes) and 0.16 mm in diameter for the ground electrode. Insulation was carried out with heat-shrink tubing (1.5/0.5 mm); the length of the

recording (uninsulated) part was ~1 mm. All electrodes were joined in a socket on a BLS-8 cable (Connfly Electronic Co. Ltd., PRC) with a pitch of 2.54 mm.

The stages of electrode implantation and postoperative animal care procedures were described in detail in a previously published paper [7]. Tiletamine/zolazepam 50 (Zoletil, Virbac, France; 10 mg/kg, intramuscularly) was used to anesthetize the animals. Electrodes FP1 and FP2 were placed in the area of primary motor cortex (AP = 0.0, ML = 2.5, DV = 1.0), C3 and C4—primary somatosensory cortex over the hippocampus (AP = –4.0, ML = 2.5, DV = 1.0), O1 and O2—secondary visual cortex (AP = –7.0, ML = 2.5, DV = 1.0). The reference electrode was implanted in the nasal bone, and the ground electrode was implanted subcutaneously in the neck region.

After the surgery rats were kept in individual cages with ad libitum access to water and food during the whole period of the study. The condition of the animals was evaluated immediately after coming out of anesthesia and then daily in the morning and evening; if necessary, the sutures were treated with iodine solution. Bicillin-3 (Sintez, Russia; 5000 U/kg, subcutaneously) was administered immediately after the surgery to prevent infection, and ketoprofen (Velpharm, Russia; 2.5 mg/kg, subcutaneously once a day for 3 days) was administered to relieve postoperative pain. To avoid dehydration, the rats were injected subcutaneously with normal saline (Grotex LLC, Russia; 5 mL once a day) during the first 3 days after the surgery.

ECoG recording in animals was performed not earlier than 7 days after the surgery using an 8-channel encephalograph Neuron-Spectrum-1 (Neurosoft, Russia) with a bandwidth of 0.5–35 Hz and a sampling frequency of 500 Hz. Signal recording was carried out simultaneously with video recording of behavior in home cage conditions under artificial light. The recording duration was 1 h and included 30 min of baseline activity (before drug or normal saline injection) and 30 min after injection. For further analysis, two 60-second samples of the recording were taken: immediately before injection and 20 min after. The selected samples contained ECoG recordings in a quiet awake state, since it has been previously shown that motor activity (locomotion, rearing, grooming or scratching) can significantly complicate the detection and differentiation of drug effects on brain bioelectrical activity in rats [8].

All the drugs used were administered intraperitoneally, if necessary pre-dissolved in physiological solution to the required concentration. The comparison drugs against which the pharmacological effects of the studied antipsychotic agents were determined during the work were haloperidol, tropicamide, chloropyramine, dexmedetomidine and phenazepam. Haloperidol (Velpharm LLC, Russia) was administered at doses of 0.3 and 2 mg/kg. The first dose was chosen as a "classical antipsychotic", blocking the effects of the dopaminomimetics apomorphine and quinpirole, as well as the 5-$HT1_B$-serotonin receptor agonist RU24969, causing hyperlocomotion and stereotypy in rats [9]. At 2 mg/kg haloperidol induces reversible catalepsy in rats [10], which allowed it to be used in this study as a marker of early extrapyramidal disorders, which are more characteristic of typical neuroleptics. In addition, haloperidol has a rather high selectivity towards dopamine D_2 receptors compared to serotonin $5HT_{2A}$-, histamine H_1-, and M-cholinoreceptors (K_i = 36 nM, 1890 nM, and >20000 nM, respectively) [11]. This selectivity explains its pronounced antipsychotic effect with a minimum of sedation and argues for its rationality of use as a reference antipsychotic.

Tropicamide (Tocris Bioscience, UK) used as a marker of antimuscarinic (atropine-like) action was administered in three doses: 0.5, 5 and 30 mg/kg [12]. The H_1-histamine receptor blocker chloropyramine (CJSC "Pharmaceutical Plant EGIS", Hungary) was used only at one dose—20 mg/kg—due to the fact that lower doses had no pronounced effect on ECoG parameters in rats, and higher doses can initiate seizure activity [13]. The α_2-adrenomimetic dexmedetomidine and the allosteric $GABA_A$ receptor modulator phenazepam were administered as reference drugs with marked sedative effects, because such action has been described for many antipsychotics as a side effect. Dexmedetomidine was used at doses of 0.005 and 0.1 mg/kg (moderate and pronounced sedation, respectively [14]), while phenazepam was used at doses of 0.1 and 1 mg/kg (sedation

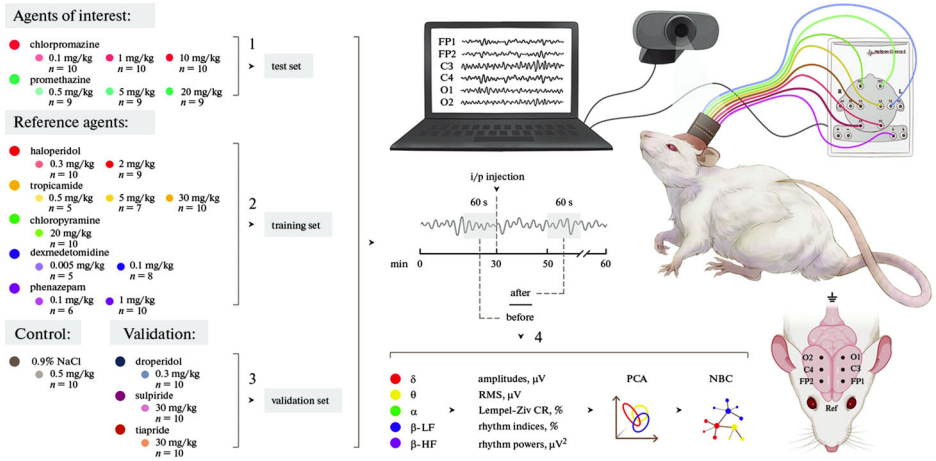


Fig. 1. Experimental design. (1) Test set: chlorpromazine and promethazine at three doses each. (2) Training set: haloperidol, tropicamide, chloropyramine, dexmedetomidine, and phenazepam. (3) Validation set: droperidol, sulpiride, and tiapride. (4) ECoG Corticographic electrodes implantation, electrocorticogram acquisition under home cage conditions with simultaneous video registration of behaviour, and analysis of selected corticogram fragments with the subsequent use of principal component analysis and the naïve Bayes classifier. I/p—intraperitoneal, RMS—root mean square, CR—compression ratio, LF—low-frequency, HF—high-frequency, PCA—principal component analysis, NBC—naïve Bayes classifier, Ref—reference electrode.

and hypnotic effects, respectively [15, 16]). Despite the fact that both dexmedetomidine and phenazepam are characterized by a pronounced sedative effect, their features of influence on ECoG parameters in rats are clearly different. In addition, the peculiarity of high doses of fenazepam is a hypnotic effect, which is not characteristic of dexmedetomidine [16, 17].

Chlorpromazine (Valenta Pharm JSC, Russia) was administered at doses of 0.1, 1 and 10 mg/kg, promethazine (CJSC "Pharmaceutical Plant EGIS", Hungary)—0.5, 5 and 20 mg/kg. The doses of both drugs were selected based on literature data [18, 19] with adjustments after our own previous studies [6, 20]. For additional validation of the obtained results, recordings with the administration of droperidol (0.3 mg/kg, FSUE "Moscow Endocrine Plant", Russia), tiapride (30 mg/kg, JSC "Organika", Russia) and sulpiride (30 mg/kg, JSC "Organika", Russia) obtained in previous work [6] were tested. As a control, normal saline was administered (Grotex LLC, Russia) at the volume of 0.5 mL.

For each drug, 5–10 recordings were made in different animals. A new drug was administered not earlier than 3 days after the previous recording to exclude interactions and residual effects. The frequency of testing of one or another rat was determined by the safety of the headplug (connector with electrodes), as well as the general condition of the animal. In the case of signs of infectious disease, Bicillin-3 (Syntez OJSC, Russia; 5000 U/kg, intramuscularly) was re-injected and the next testing was performed not earlier than one week after the injection.

The obtained recordings were analyzed using the program Neuron-Spectrum.NETomega 1.6.10.8 (Neurosoft LLC, Russia). For all 6 channels (FP1, FP2, C3, C4, O1 and O2), a total of 132 amplitude-spectral characteristics of ECoG were calculated, including mean and maximum signal amplitudes, standard deviation and Lempel-Zivcompression ratio, mean amplitudes of wave rhythms, indices and mean power of rhythms. The δ- (0.5–4.0 Hz), θ- (4.0–8.0 Hz), α- (8.0–14.0 Hz) and β-rhythms (low-frequency (LF)—14.0–20.0 Hz and high-frequency (HF)—20.0–35.0 Hz) were extracted from the signal. The data were expressed as ratios of parameter values after drug administration to the values of the corresponding parameters before administration (Fig. 1).

Processing and subsequent analysis of the obtained data were performed using the add-in for MS Excel XLSTAT 2016.02.28451 and sklearn v1.1.2 module. Data dimensionality reduction

was performed using the principal component analysis (PCA), and then the NBC model was trained on the basis of the extracted principal components. The training set included data from records of the effects of haloperidol, tropicamide, chloropyramine, dexmedetomidine and phenazepam.

Quality assessment of the NBC model on the training set was performed using 10-fold repetition of randomized cross-validation with stratification by groups of 5 folds, function 'RepeatedStratifiedKFold(n_splits=5, n_repeats=10)' in sklearn. In this case, the entire training set is divided randomly into 5 blocks while maintaining the proportions of each drug in the group, then training is performed on each of the 4 blocks and quality check on the fifth, then the procedure is repeated 10 times. This approach provides a consistent quality score that is independent of the particular partitioning. Quality was evaluated as the total percentage of correctly classified drugs and using an adjacency matrix for classification results relative to correct answers, to detail classification errors. The model was then evaluated on the whole training set and was used to predict the pharmacological activity of the experimental drugs. A scatter plot for the first four principal components was used to visualize the differences in the distribution of principal components for the administered drugs. Also, formal tests for differences of the study drugs relative to saline solution were performed for the first four principal components. The first component was chosen as containing most of the variability in the data and was the most informative in terms of differences. Four components were also used in training the classifier.

The significance of differences between the groups in Fig. 4 when comparing two groups was assessed using Student's *t*-criterion (for normal distribution and equality of variances) and Mann–Whitney criterion (for distribution other than normal/difference of variances). When comparing the four groups, one-factor analysis of variance with Tukey's test (when the distribution was normal and variability of differences was equal), or the Kruskel–Wallis test with Dunn's *post hoc* test (when the distribution was non-normal/variability of differences was not equal) were used. The Shapiro–Wilk *W*-criterion was used to test the normality of data distribution, and the Bartlett test was used to assess the equality of differences.

RESULTS

Administration of the investigated drugs resulted in changes in the amplitude-spectral characteristics of ECoG in the test animals. The patterns of changes in the mean power of δ-, θ-, α- and β-rhythms in all 6 recording channels are shown as an example (Fig. 2). It should be noted that not always increasing the dose of the administered drug resulted in a linear increase in the average power of rhythms, as well as in the number of channels where such an increase was recorded. For example, if at a dose of 1 mg/kg chlorpromazine increased the average power of θ-, α-, and β-LF rhythms in frontal channels, as well as the power of α- and β-LF rhythms in occipital channels, then a 10-fold increase in the dose of the drug had less effect on channels O1 and O2. Similarly, the 5 mg/kg dose of promethazine increased the activity of δ rhythms in all cortical areas in rats to a lesser extent than 0.5 mg/kg, although in general the pattern of ECoG changes under the influence of both doses was similar. In most cases, administration of one or another drug resulted in an increase in the average power of the recorded rhythms, except for chloropyramine, which decreased the activity of α-, β-LF, and β-HF rhythms. Chloropyramine, sulpiride, and tiapride had the least pronounced effects on brain bioelectrical activity in rats, but the nature of their action differed from the changes observed after administration of physiological solution (a slight increase in δ-activity with a decrease in the power of θ-rhythms).

Analysis of the obtained PCA data showed that 77.05% of the variance can be described by 4 principal components (PC1–PC4), which were used for further analysis. Factor loadings of the analyzed ECoG parameters characterizing their contribution to the formation of one or another principal component are shown in Fig. 3. The PC1 component, describing 48.8% of the dispersion, was formed by the amplitude characteristics of the signal (both in general and individual rhythms), as well as the values of the spectral

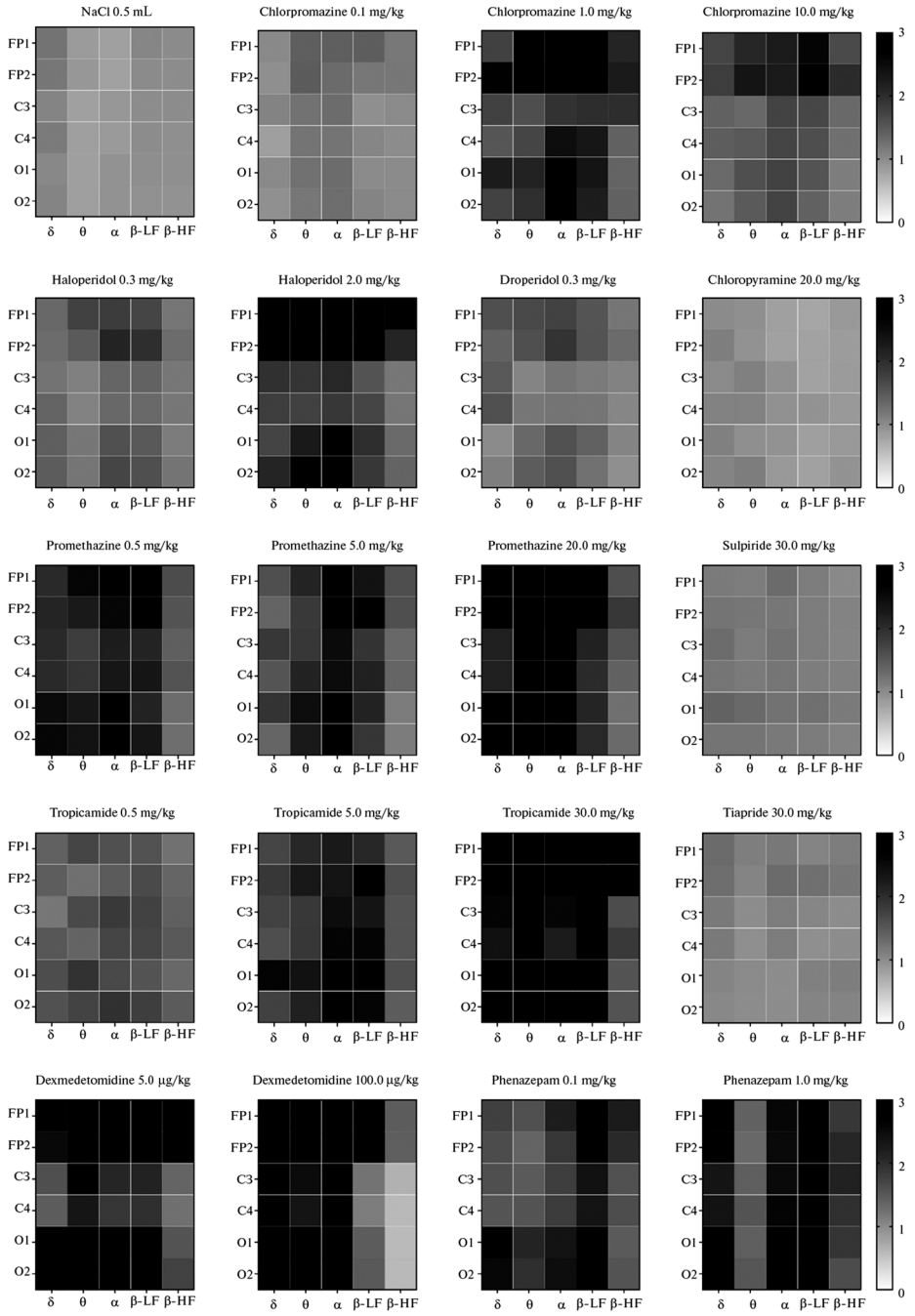
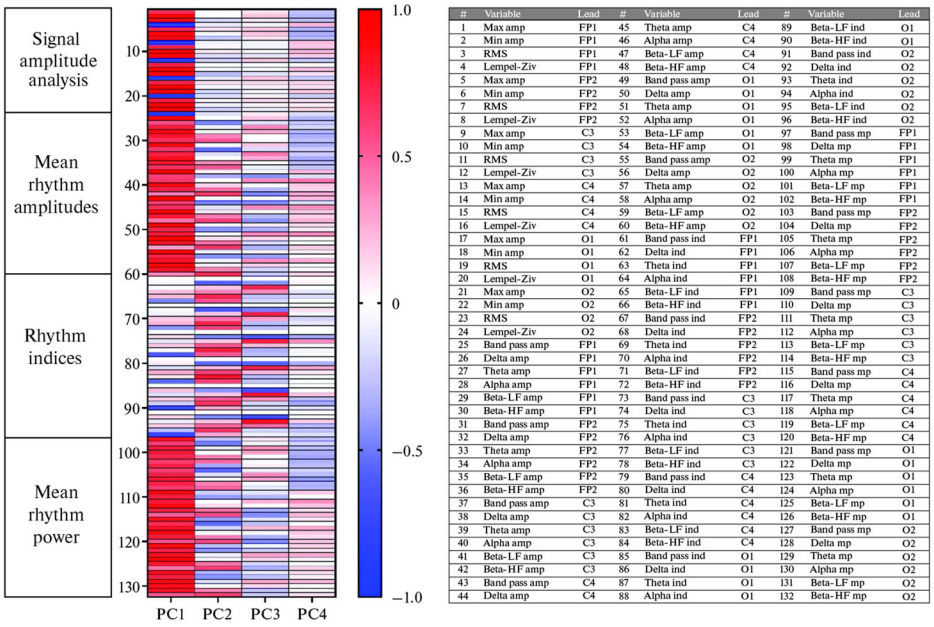


Fig. 2. Heatmaps of median values (n = 5–10 for each group) of mean rhythm powers for the δ-, θ-, α- and β-rhythms in channels FP1, FP2, C3, C4, O1, and O2 following drug administration. The heatmaps reflect the ratios of mean rhythm power values at 20 min following drug administration to the respective values before the administration (30th minute into the baseline record).

power of rhythms correlated with them. All the above parameters of ECoG influenced the value of PC1 regardless of the location of recording electrodes. The value of PC2 component (14.1%) was influenced to a greater extent by the activity of δ- and β-rhythms in all channels. The value of PC3 (9.43%) was almost completely determined by the indices of δ- and θ-rhythms. The PC4 component (4.75%) was formed mainly by the magnitude of the average amplitude and power of the θ-rhythm in parietal and occipital cortical areas.



#	Variable	Lead	#	Variable	Lead	#	Variable	Lead
1	Max amp	FP1	45	Theta amp	C4	89	Beta-LF ind	O1
2	Min amp	FP1	46	Alpha amp	C4	90	Beta-HF ind	O1
3	RMS	FP1	47	Beta-LF amp	C4	91	Band pass ind	O2
4	Lempel-Ziv	FP1	48	Beta-HF amp	C4	92	Delta ind	O2
5	Max amp	FP2	49	Band pass amp	O1	93	Theta ind	O2
6	Min amp	FP2	50	Delta amp	O1	94	Alpha ind	O2
7	RMS	FP2	51	Theta amp	O1	95	Beta-LF ind	O2
8	Lempel-Ziv	FP2	52	Alpha amp	O1	96	Beta-HF ind	O2
9	Max amp	C3	53	Beta-LF amp	O1	97	Band pass mp	FP1
10	Min amp	C3	54	Beta-HF amp	O1	98	Delta mp	FP1
11	RMS	C3	55	Band pass amp	O2	99	Theta mp	FP1
12	Lempel-Ziv	C3	56	Delta amp	O2	100	Alpha mp	FP1
13	Max amp	C4	57	Theta amp	O2	101	Beta-LF mp	FP1
14	Min amp	C4	58	Alpha amp	O2	102	Beta-HF mp	FP1
15	RMS	C4	59	Beta-LF amp	O2	103	Band pass mp	FP2
16	Lempel-Ziv	C4	60	Beta-HF amp	O2	104	Delta mp	FP2
17	Max amp	O1	61	Band pass ind	FP1	105	Theta mp	FP2
18	Min amp	O1	62	Delta ind	FP1	106	Alpha mp	FP2
19	RMS	O1	63	Theta ind	FP1	107	Beta-LF mp	FP2
20	Lempel-Ziv	O1	64	Alpha ind	FP1	108	Beta-HF mp	FP2
21	Max amp	O2	65	Beta-LF ind	FP1	109	Band pass mp	C3
22	Min amp	O2	66	Beta-HF ind	FP1	110	Delta mp	C3
23	RMS	O2	67	Band pass ind	FP2	111	Theta mp	C3
24	Lempel-Ziv	O2	68	Delta ind	FP2	112	Alpha mp	C3
25	Band pass amp	FP1	69	Theta ind	FP2	113	Beta-LF mp	C3
26	Delta amp	FP1	70	Alpha ind	FP2	114	Beta-HF mp	C3
27	Theta amp	FP1	71	Beta-LF ind	FP2	115	Band pass mp	C4
28	Alpha amp	FP1	72	Beta-HF ind	FP2	116	Delta mp	C4
29	Beta-LF amp	FP1	73	Band pass ind	C3	117	Theta mp	C4
30	Beta-HF amp	FP1	74	Delta ind	C3	118	Alpha mp	C4
31	Band pass amp	FP2	75	Theta ind	C3	119	Beta-LF mp	C4
32	Delta amp	FP2	76	Alpha ind	C3	120	Beta-HF mp	C4
33	Theta amp	FP2	77	Beta-LF ind	C3	121	Band pass mp	O1
34	Alpha amp	FP2	78	Beta-HF ind	C3	122	Delta mp	O1
35	Beta-LF amp	FP2	79	Band pass ind	C4	123	Theta mp	O1
36	Beta-HF amp	FP2	80	Delta ind	C4	124	Alpha mp	O1
37	Band pass amp	C3	81	Theta ind	C4	125	Beta-LF mp	O1
38	Delta amp	C3	82	Alpha ind	C4	126	Beta-HF mp	O1
39	Theta amp	C3	83	Beta-LF ind	C4	127	Band pass mp	O2
40	Alpha amp	C3	84	Beta-HF ind	C4	128	Delta mp	O2
41	Beta-LF amp	C3	85	Band pass ind	O1	129	Theta mp	O2
42	Beta-HF amp	C3	86	Delta ind	O1	130	Alpha mp	O2
43	Band pass amp	C4	87	Theta ind	O1	131	Beta-LF mp	O2
44	Delta amp	C4	88	Alpha ind	O1	132	Beta-HF mp	O2

Fig. 3. Factor loadings reflecting the degree of involvement of each of the 132 parameters analyzed into the formation of the PC, PC2, PC3, and PC4 principal components, used for the subsequent analysis using the naïve Bayes classifier. Amp—amplitude (μV), RMS—root mean square (μV), Lempel-Ziv—Lempel-Ziv compression ratio (%), ind—rhythm index (%), mp—mean rhythm power (μV^2).

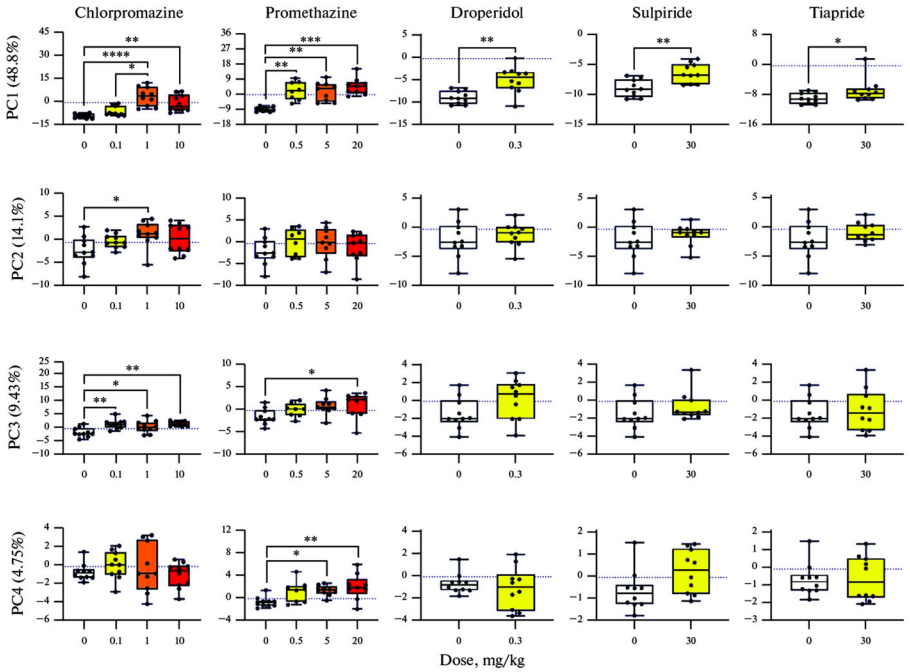


Fig. 4. PC1–PC4 principal component values for the chlorpromazine (0.1, 1, and 10 mg/kg), promethazine (0.5, 5, and 20 mg/kg), droperidol, sulpiride, and tiapride. For control (white boxplots), we used principal component values obtained for the group receiving 0.5 mL of saline. Data are shown as median (min; max). Here, we made the adjustment for multiple comparisons when comparing the doses of one agent (chlopromazine or promethazine), but did not take that into account when testing simultaneously the four principal components or the five other drugs, as we conducted those comparisons solely for the purpose of data visualization. * $p < 0.05$, ** $p < 0.01$.

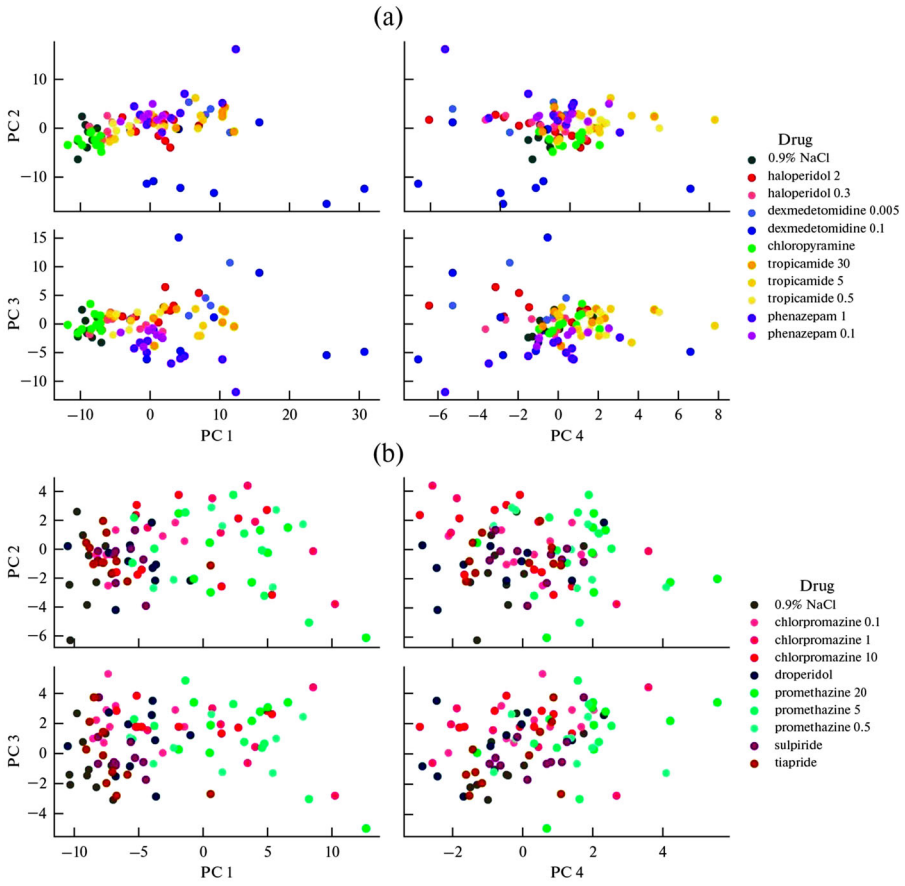


Fig. 5. Scatterplots for the first four principal components obtained for the training set (a), and projections of the test set agents on these components (b).

When comparing the effects of the studied drugs on the magnitude of the PC1–PC4 components, it was noted that all of them at all administered doses, except chlorpromazine at a dose of 0.1 mg/kg, caused a statistically significant increase in the first principal component compared to the control ($p < 0.05$ for tiapride and $p < 0.01$ for chlorpromazine at doses of 1 and 10 mg/kg, promethazine at doses of 0.5, 5 and 20 mg/kg, droperidol and sulpiride, respectively) (Fig. 4). Only chlorpromazine at a dose of 1 mg/kg had an effect on PC2 values ($p < 0.05$). Component PC3 values were significantly increased by chlorpromazine at all doses tested ($p < 0.05$ for the 1 mg/kg dose and $p < 0.01$ for the 0.1 and 10 mg/kg doses) and promethazine at the 20 mg/kg dose ($p < 0.05$). PC4 was higher only in animals administered promethazine at doses of 5 and 20 mg/kg ($p < 0.05$ and $p < 0.01$, respectively).

Figure 5 shows the scatter plots for the first four principal components on the training (a) and test set (b). Physiological solution was also added to the test set to show that the study drugs lie far enough away from it in the principal component space.

The confusion matrix with classification results for the training set is shown in Fig. 6.

Next, the drugs from the test and validation sets were projected onto the principal components from the training set, and then the pharmacological activity of the analyzed records was predicted for the obtained data on the basis of principal component values using the trained NBC. For each record, the probabilities of matching effects on ECoG with those of the drugs used as a training set (haloperidol, tropicamide, chloropyramine, dexmedetomidine, and phenazepam) were calculated. For all groups tested, the median value of the probability of similarity to a reference group was calculated, from which conclusions could be

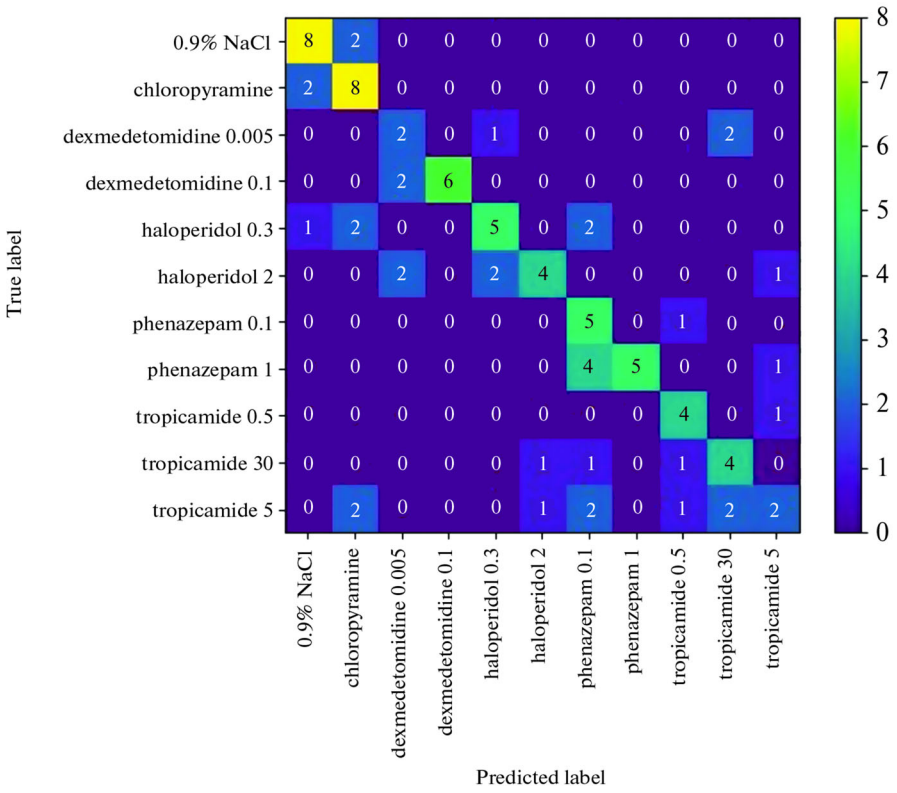


Fig. 6. Results of the training set classification by the naïve Bayes classifier.

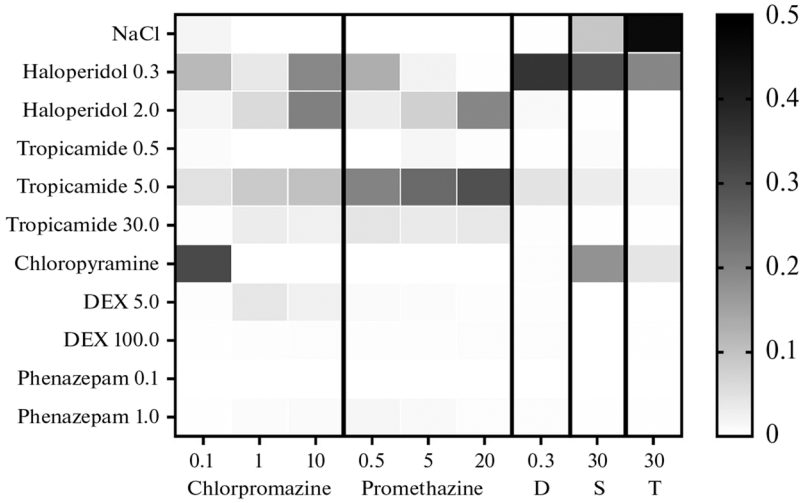


Fig. 7. A heatmap for the median identity probability values for the pharmacological effects of chlorpromazine (0.1, 1, and 10 mg/kg), promethazine (0.5, 5, and 20 mg/kg), droperidol (D), sulpiride (S), and tiapride (T) and the training set drugs (vertical axis), obtained with the use of the naïve Bayes classifier.

drawn about the nature of action of chlorpromazine and promethazine at a particular dose tested (Fig. 7). To further validate the methodology, pharmacological activity was predicted for droperidol (0.3 mg/kg), sulpiride (30 mg/kg), and tiapride (30 mg/kg).

Chlorpromazine at a low dose of 0.1 mg/kg was found to have the highest similarity to chloropyramine and haloperidol 0.3 mg/kg (median similarity probability values of 0.308 and 0.113,

respectively), which may indicate the predominance of H_1-antihistamine and antipsychotic effects of the drug at this dose. The 10 mg/kg dose also showed similarity to the antipsychotic dose of haloperidol (0.196). At the same time, NBC predicted a pronounced "cataleptogenic" effect (similarity to haloperidol at a dose of 2.0 mg/kg, median similarity probability value of 0.206). All three doses of chlorpromazine produced effects similar to those of tropicamide at a dose of 5 mg/kg. There was a dose-dependence of such antimuscarinic effects (0.049, 0.085, and 0.101 for doses of 0.1, 1, and 10 mg/kg, respectively). Also, some similarity to dexmedetomidine at the 5 mg/kg dose was predicted for the medium and high doses of chlorpromazine (0.041 and 0.024, respectively), which may suggest their moderate sedative action.

Promethazine at all administered doses was defined by the NBC as primarily an antimuscarinic (median probability of similarity to tropicamide 5 mg/kg was 0.201, 0.247, and 0.295 for doses of 0.5, 5, and 20 mg/kg, respectively). Also for this drug, a clear predicted dose-dependent transition from antipsychotic to cataleptogenic action was found (similarities with haloperidol 0.3 mg/kg were 0.131, 0.020 and 0.001, and with haloperidol 2 mg/kg were 0.031, 0.075 and 0.198 for doses of 0.5, 5 and 20 mg/kg, respectively). Interestingly, for promethazine, the proposed approach failed to detect any pronounced H_1-antihistamine effect at any of the doses studied.

Droperidol at the only dose tested was defined as an "antipsychotic" agent (similarity to haloperidol 0.3 mg/kg 0.353) with a weak "antimuscarinic" effect (similarity to tropicamide 5 mg/kg 0.047). Sulpiride and tiapride were categorized as "antipsychotics" with weak to moderate H_1-antihistamine effects. However, it is worth noting that for both drugs, a rather high similarity was shown with the control group administered saline (0.091 and 0.459 for sulpiride and tiapride, respectively).

DISCUSSION

It has previously been shown that NBC in combination with PCA can be used to compare the effects of antipsychotic agents, as well as their differences from the benzodiazepine tranquilizer phenazepam, which has sedative activity [6]. Nevertheless, it is well known that antipsychotics can demonstrate different pharmacological effects depending on the administered dose. This is largely due to the inselective receptor action of these drugs. For most of them, the most frequent secondary pharmacological targets are muscarinic receptors, H_1-histamine receptors, α-adrenoreceptors and various subtypes of serotonin receptors [11]. Therefore, one of the key steps in the development of the proposed approach as a pharmacological screening tool is to record the effects of reference drugs in different dose ranges. Despite the obviousness of this statement, in the works of other authors [21, 22], using pharmaco-EEG for screening and classification of psychologic drugs, no emphasis was placed on the dose-dependence of the recorded effects.

In the present study, the sensitivity of the pharmacological activity prediction algorithm used was evaluated to the above-mentioned dose-dependence of the effects of two selected antipsychotics, chlorpromazine and promethazine. Agents with the most classical pharmacological effects, namely antipsychotic and cataleptogenic (haloperidol), atropine-like (tropicamide), H_1-antihistamine (chloropyramine) and sedative/soporific effects (dexmedetomidine and phenazepam) were chosen as reference drugs. It should be taken into account that all the selected drugs are not selective to their main pharmacological targets: for example, haloperidol can block serotonin $5HT_{2A}$ receptors [11], and chloropyramine, being a representative of H_1-histamine blockers of the 1st generation, does not lack antimuscarinic effect [23]. In the future, a possible solution to this problem may be the use of selective agonists/antagonists of the molecular targets of interest, such as the D_2-receptor blockers raclopride, L-741626 and others. However, as a rule, there are no data on clinical effects and pharmacokinetics for such molecules; moreover, over time, a detailed study of the pharmacodynamics of a particular ligand reveals new pharmacological targets, and it is no longer considered selective. The most promising is the sequential use of two training sets, "by the effect" and "by the receptor", but this approach requires experimental validation.

Using NBC, it was revealed that chlorproma-

zine at a low dose of 0.1 mg/kg has antipsychotic and H_1-antihistamine effects, at a dose of 1 mg/kg—antipsychotic, and at a dose of 10 mg/kg the cataleptogenic effect becomes noticeable (Fig. 5). In previously published work by other authors [24] it was shown that chlorpromazine in doses of 0.1, 1 and 5 mg/kg can reduce emotional hyperthermia in rats in the "resident-intruder" test (statistically significant for the dose of 5 mg/kg and at the trend level—for doses of 0.1 and 1 mg/kg). This test is sensitive to the effects of a number of neuroleptics and is considered promising for assessing antipsychotic effects. According to Rebec et al., chlorpromazine has a moderate cataleptogenic effect in rats at doses of 5 and 10 mg/kg [25]. Thus, the dose-dependent antipsychotic and cataleptogenic effects of chlorpromazine predicted in the present work are consistent with the literature data.

Based on literature data, it is not possible to compare the predicted dose-dependence of the antimuscarinic and H_1-antihistamine effects of chlorpromazine. Nevertheless, there is no doubt that these effects should increase with increasing dose of the administered antipsychotic. In the present study, only a single, low dose of chlorpromazine was rated as "antihistamine", while the "atropine-like" effects increased from a dose of 0.1 mg/kg to a dose of 10 mg/kg. The most likely explanation for this phenomenon may be that the notable antimuscarinic effect masks the H_1-antihistamine action on ECoG in rats. Figure 2 clearly shows how weakly pronounced the effects of chloropyramine are even at a fairly high administered dose (20 mg/kg) compared with three doses of tropicamide. Also in favor of masking of antihistamine effect by antimuscarinic effect are the results obtained for 3 doses of promethazine (Fig. 5).

Promethazine is currently used as an antiemetic and anti-allergic agent, as well as for premedication because of its ability to potentiate anesthesia and induce artificial hibernation. As a phenothiazine derivative with a dopamine-like effect, this drug was seriously considered in the 50s and 60s as an antipsychotic, but a double-blind placebo-controlled study conducted in 1960 showed greater efficacy of another phenothiazine derivative, chlorpromazine, for the treatment of schizophrenia [26]. Because of this, and also because of the appearance at the same time of butyrophenone derivatives (and later second- and third-generation antipsychotics) [27], interest in promethazine as an antipsychotic will finally fade. In the present work, this drug is not regarded as an underestimated antipsychotic, but serves only as a means of validating the proposed methodology. As with chlorpromazine, promethazine demonstrated a dose-dependent transition from "antipsychotic" to "cataleptogenic" action (Fig. 5), which is characteristic of typical antipsychotics. Knowing in advance that chlorpromazine is a stronger antipsychotic than promethazine, it can be concluded that the calculated median value of the probability of similarity to haloperidol at a dose of 0.3 mg/kg is not a quantitative characterization of the strength of the antipsychotic effect that can be compared between the different drugs studied. Nevertheless, for all doses of promethazine (especially for 5 and 20 mg/kg) a marked predominance of "antimuscarinic" effect over "antipsychotic" (or even "cataleptogenic") effect was shown; this indirectly suggests that the drug under study is primarily an M-choline blocker, and only to some extent a dopamine D_2-receptor antagonist. This observation is fully consistent with the previously published values of inhibition constants (K_i, nM) reflecting affinity to dopamine and M-cholinoreceptors (Table 1).

Droperidol was defined as a "classical" antipsychotic with a weak atropine-like effect. As in the case of promethazine, the data obtained are consistent with K_i values for D_2-dopamine and M-cholinoreceptors (Table 1). The atypical antipsychotics sulpiride and tiapride, on the one hand, were classified as antipsychotics by the NBC; on the other hand, their high similarity to saline and the H_1-histamine receptor blocker chloropyramine was demonstrated. The similarity with haloperidol at a dose of 0.3 mg/kg is expected and once again demonstrates the sensitivity of the proposed method for detecting antipsychotic action. Nevertheless, the other two predictions may raise some questions to the correctness of the algorithm. On the one hand, the similarity with normal saline and chloropyramine may be due to the fact that both atypical antipsychotics and the H_1-histamine receptor antagonist induced weak ECoG changes

Table 1. Inhibition constant values (K_i, nM), reflecting the affinity of chlorpromazine, promethazine, droperidol, tiapride, and sulpiride towards different receptors

Agent	D_2-DR	M-ChR	H_1-HR
Chlorpromazine	11.0 [28] 19.0 [11]	1.5 [28] 60.0 [11]	25.0 [28] 9.1 [11]
Promethazine	260.0 [29]	3.32 (M_1) 12.0 (M_2) 4.15 (M_3) 1.06 (M_4) 3.31 (M_5) [29]	0.24 [30] 0.33 [29]
Droperidol	0.25 [31] 0.8 [32]	537.0 (M_4) 1651.0 (M_5) [32]	525.0 [32]
Sulpiride	8.2 [33] 13.0 [28]	> 1300 [28]	> 80000 [28]
Tiapride	226.0 [33]	NA	NA

DR—dopamine receptor, ChR—cholinergic receptor, HR—histamine receptor, NA—not available.

in rats, thus any drugs that have little effect on the cortex would be considered similar to each other or to the control group. This statement is not unfair given the fact that chlorpromazine at a dose of 0.1 mg/kg still had some similarity to NaCl (Fig. 5). On the other hand, this same low dose of chlorpromazine was clearly identified as "antihistamine" and "antipsychotic". A possible solution to this problem in the future may be the use of other H_1-histamine receptor blockers that will better penetrate the blood–brain barrier, have greater affinity for the receptors of interest, and due to this will modify the amplitude-spectral characteristics of the ECoG to a greater extent. As part of this work, recordings were also made of the effects of diphenhydramine at a dose of 20 mg/kg (data not presented), but the effects of this drug were not more pronounced than those of chloropyramine.

Weak ECoG effects of some drugs may be a serious problem for solving further prediction problems using the proposed method. The use of different variations of combinations of dimensionality reduction algorithms (PCA, LDA, etc.) and machine learning methods (NBC, KNN, SVM, etc.) is likely to allow more efficient separation of such drugs from each other. It should also be noted that the present study was performed on healthy animals, but under pathological conditions the effects of drugs may not only become more pronounced, but also change. In this regard, it is of interest to conduct pharmaco-EEG studies on experimental models (genetic, pharmacological, etc.) of CNS diseases.

Another possible approach (not excluding the first two options) could be the use of depth electrodes to record the effects of drugs on brain bioelectrical activity in rats. For example, Dimpfel in his works used signals recorded not only from the cerebral cortex, but also from the striatum, hippocampus, and reticular formation [4, 5]. However, this approach requires a longer time of electrode manufacturing, a longer procedure of surgical implantation, as well as additional histological validation of the electrode location after the animals are removed from the experiment, which does not allow it to be considered as a tool for quick and simple pharmacological screening.

Predicting the similarity of the studied drugs with dexmedetomidine and phenazepam deserves special attention. As it was mentioned earlier, both drugs have sedative action, nevertheless, the induced sedation is different both in terms of mechanisms of action and in the nature of the effect on ECoG in rats. To a greater extent, the sedative action induced by typical antipsychotics is commonly associated with blockade of H_1-his-

tamine receptors [11, 34]. Therefore, it seems logical that the antipsychotics considered in the present study showed very little similarity to the α_2-adrenomimetic dexmedetomidine and the $GABA_A$ receptor modulator phenazepam. On the other hand, both drugs still have some affinity to α_2-adrenoreceptors. Chlorpromazine at high doses (1 and 10 mg/kg) showed similarity to dexmedetomidine at a dose of 5 mg/kg, while promethazine showed almost no similarity (the K_i of chlorpromazine for α_2-adrenoreceptors according to literature data is 750 nM, and for promethazine, depending on the receptor subtype—256, 34, 353 nM, for A-, B- and C-subtypes, respectively). Probably, in this case, the ratio of K_i receptors within one particular ligand plays a major role, and in the case of promethazine its α_2-adreno-blocking effect, as well as H_1-antihistamine effect, may overlap with atropine-like action.

Summarizing the results, we can conclude that NBC in combination with PCA can be used to reveal the dose-dependence of antipsychotic drug effects by their influence on ECoG parameters in rats. The proposed approach makes it possible to evaluate the severity of antipsychotic, cataleptogenic, atropine-like (antimuscarinic), H_1-antihistamine, sedative or hypnotic effects of the studied drugs in different dose ranges. The weakness of the action of some psychotropic drugs (e.g., H_1-histamine receptor blockers, as well as the benzamide derivatives sulpiride and tiapride) may require additional methodological approaches, as which more advanced classification and prediction algorithms, animals with disease models or with depth electrodes may be used.

AUTHORS' CONTRIBUTION

Idea of work and planning the experiment (Yu.I.S., S.V.O.), experimentation and data processing (Yu.I.S., M.V.S., D.D.Sh., M.M.P., I.S.K., M.S.K.), preparing illustrations (Yu.I.S., V.A.P., I.S.K., M.S.K.), writing and editing the manuscript (Yu.I.S., V.A.P., I.A.T., N.O.S., S.V.O.).

FUNDING

This work was supported by Russian Science Foundation grant 23-75-01051 (creation of a library of electrocorticogram records), State Program GP-47 "Scientific and Technological Development of the Russian Federation" (2019–2030), topic 0113-2019-0006 (Yu.I.S.), under the projects 93022925/94030803 St. Petersburg State University (Yu.I.S.) and no. 075-10-2021-093 (Project NRB-RND-2115, Yu.I.S.) with the financial support of the Ministry of Science and Higher Education of the Russian Federation, as well as at the expense of the state assignment of the N.P. Bechtereva Institute of the Human Brain (development of methods of mathematical analysis, topic state registration number 122041500045-8).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Experiments using laboratory animals were performed in accordance with the requirements of Directive 2010/63/EU of the European Parliament and Council of September 22, 2010, the principles of the Basel Declaration and the requirements of the Council of the Eurasian Economic Union from November 03, 2016 no. 81 "On Approval of the Rules of Good Laboratory Practice of the Eurasian Economic Union in the field of circulation of medicines". The protocol of the experiment was approved by the bioethical commission of Saint Petersburg State Chemical and Pharmaceutical University of the Ministry of Health of Russia (protocol-application R-PEEG2-SA-2022 dated February 15, 2022). All measures were taken to reduce the number of animals used and minimize their suffering.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest

REFERENCES

1. Lanzone J, Ricci L, Tombini M, Boscarino M, Mecarelli O, Pulitano P, Di Lazzaro V, Assenza G (2021) The effect of Perampanel on EEG spectral power and connectivity in patients with focal epilepsy. Clin Neurophysiol 132(9): 2176–2183.

https://doi.org/10.1016/j.clinph.2021.05.026
2. Hyun J, Baik MJ, Kang UG (2011) Effects of Psychotropic Drugs on Quantitative EEG among Patients with Schizophrenia-spectrum Disorders. Clin Psychopharmacol Neurosci 9(2): 78–85. https://doi.org/10.9758/cpn.2011.9.2.78
3. Nordin C, Krijzer F (1996) Antidepressant and anxiolytic profiles of E-10-hydroxynortriptyline on electrocorticograms of rats. Neuropsychobiology 34(1): 44–48. https://doi.org/10.1159/000119290
4. Dimpfel W (2007) Characterization of atypical antipsychotic drugs by a late decrease of striatal alpha1 spectral power in the electropharmacogram of freely moving rats. Br J Pharmacol 152(4): 538–548. https://doi.org/10.1038/sj.bjp.0707427
5. Dimpfel W, Hoffmann JA (2010) Electropharmacograms of rasagiline, its metabolite aminoindan and selegiline in the freely moving rat. Neuropsychobiology 62(4): 213–220. https://doi.org/10.1159/000319947
6. Sysoev YuI, Shits DD, Puchik MM, Prikhodko VA, Idiyatullin RD, Kotelnikova AA, Okovityi SV (2022) Use of Naïve Bayes Classifier to Assess the Effects of Antipsychotic Agents on Brain Electrical Activity Parameters in Rats. J Evol Biochem Physiol 58(4): 1130–1141. https://doi.org/10.1134/S0022093022040160
7. Sysoev YuI, Prikhodko VA, Idiyatullin RD, Chernyakov RT, Karev VE, Okovityi SV (2022) A Method for Chronic Registration of Brain Cortical Electrical Activity in Rats. J Evol Bioch Physiol 58: 292–301. https://doi.org/10.31857/S0869813922020091
8. Hansen IH, Agerskov C, Arvastson L, Bastlund JF, Sørensen HBD, Herrik KF (2019) Pharmacoelectroencephalographic responses in the rat differ between active and inactive locomotor states. Eur J Neurosci 50(2): 1948–1971. https://doi.org/10.1111/ejn.14373
9. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 156(2–3): 117–154. https://doi.org/10.1007/s002130100811
10. Waku I, Magalhães MS, Alves CO, de Oliveira AR (2021) Haloperidol-induced catalepsy as an animal model for parkinsonism: A systematic review of experimental studies. Eur J Neurosci 53(11): 3743–3767. https://doi.org/10.1111/ejn.15222
11. Gardner DM, Baldessarini RJ, Waraich P (2005) Modern antipsychotic drugs: a critical overview. CMAJ 172(13): 1703–1711. https://doi.org/10.1503/cmaj.1041064
12. Bryant SM, Rhee JW, Thompson TM, Lu JJ, Aks SE (2009) Parenteral ophthalmic tropicamide or cyclopentolate protects rats from lethal organophosphate poisoning. Am J Ther 16(3): 231–234. https://doi.org/10.1097/MJT.0b013e318182254b
13. Kamei C, Ohuchi M, Sugimoto Y, Okuma C (2000) Mechanism responsible for epileptogenic activity by first-generation H1-antagonists in rats. Brain Res 887(1): 183–186. https://doi.org/10.1016/s0006-8993(00)03041-9
14. Hunter JC, Fontana DJ, Hedley LR, Jasper JR, Lewis R, Link RE, Secchi R, Sutton J, Eglen RM (1997) Assessment of the role of alpha2-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice. Br J Pharmacol 122(7): 1339–1344. https://doi.org/10.1038/sj.bjp.0701520
15. Arushanyan EB, Beyer EV, Kaminskaya OV, Sotnikova LK (2015) Positive aspects of the combined effect of melatonin and phenazepam on the behavioral activity of rats. Med Herald North Caucasus 1(37): 84–88. (In Russ). https://doi.org/10.14300/mnnc.2015.10014
16. Kan AV, Prikhodko VA, Sysoev YuI, Okovity SV (2021) Effect of phenazepam on the amplitude characteristics of electrocorticogram rhythms in rats. Bull Med Sci 4(24): 108–111. (In Russ). https://doi.org/10.31684/25418475-2021-4-108
17. Garrity AG, Botta S, Lazar SB, Swor E, Vanini G, Baghdoyan HA, Lydic R (2015) Dexmedetomidine-induced sedation does not mimic the neurobehavioral phenotypes of sleep in Sprague Dawley rat. Sleep 38(1): 73–84. https://doi.org/10.5665/sleep.4328
18. Paalzow GHM, Paalzow LK (1985) Promethazine both facilitates and inhibits nociception in rats: Effect of the testing procedure. Psychopharmacology 85: 31–36. https://doi.org/10.1007/BF00427318
19. Drozdov AL, Demchenko EM, Eyad A, Nerush OP (2011) The influence of psychotropic drugs on the spontaneous behavioral activity of white rats. Visn Dnipropetrov Univer Biol Med 1(2): 47–53. (In Russ).
20. Shits DD, Puchik MM, Prikhodko VA, Sysoev YuI, Okovityi SV (2022) Effects of promethazine on the amplitude and spectral characteristics of electrocorticograms in rats. Bull Perm Univ Biol (4): 352–356. https://doi.org/10.17072/1994-9952-2022-4-

352-356
21. Krijzer F, Koopman P, Olivier B (1993) Classification of psychotropic drugs based on pharmacoelectrocorticographic studies in vigilance-controlled rats. Neuropsychobiology 28(3): 122–137. https://doi.org/10.1159/000119015
22. Dimpfel W (2003) Preclinical data base of pharmaco-specific rat EEG fingerprints (tele-stereo-EEG). Eur J Med Res 8(5): 199–207.
23. Goryachkina MV, Belousova TA (2014) Chloropyramine: clinical aspects of use. RMJ Med Review 22(24): 1785–1789. (In Russ).
24. Blessing WW, Blessing EM, Mohammed M, Ootsuka Y (2017) Clozapine, chlorpromazine and risperidone dose-dependently reduce emotional hyperthermia, a biological marker of salience. Psychopharmacology (Berl) 234(21): 3259–3269. https://doi.org/10.1007/s00213-017-4710-x
25. Rebec GV, Gelman J, Alloway KD, Bashore TR (1983) Cataleptogenic potency of the antipsychotic drugs is inversely correlated with neuronal activity in the amygdaloid complex of the rat. Pharmacol Biochem Behav 19(5): 759–763. https://doi.org/10.1016/0091-3057(83)90076-x
26. Casey JF, Bennett IF, Lindley CJ, Holister LE, Gordon MH, Springer NN (1960) Drug therapy in schizophrenia. A controlled study of the relative effectiveness of chlorpromazine, promazine, phenobarbital, and placebo. AMA Arch Gen Psychiatry 2: 210–220. https://doi.org/10.1001/archpsyc.1960.03590080086012
27. Ramachandraiah CT, Subramaniam N, Tancer M (2009) The story of antipsychotics: Past and present. Indian J Psychiatry 51(4): 324–326. https://doi.org/10.4103/0019-5545.58304
28. Yonemura K, Miyanaga K, Machiyama Y (1998) Profiles of the affinity of antipsychotic drugs for neurotransmitter receptors and their clinical implication. Kitakanto Med J 48(2): 87–102. https://doi.org/10.2974/KMJ.48.87
29. Promethazine. Ligand Activity Charts. IUPHAR/BPS Guide to Pharmacology. Mode of access: https://www.guidetopharmacology.org (access from May 29, 2023)
30. Ghoneim OM, Legere JA, Golbraikh A, Tropsha A, Booth RG (2006) Novel ligands for the human histamine H1 receptor: synthesis, pharmacology, and comparative molecular field analysis studies of 2-dimethylamino-5-(6)-phenyl-1,2,3,4-tetrahydronaphthalenes. Bioorg Med Chem 14(19): 6640–6658. https://doi.org/10.1016/j.bmc.2006.05.077
31. Li P, Snyder GL, Vanover KE (2016) Dopamine Targeting Drugs for the Treatment of Schizophrenia: Past, Present and Future. Curr Top Med Chem 16(29): 3385–3403. https://doi.org/10.2174/1568026616666160608084834
32. Droperidol. Ligand Activity Charts. IUPHAR/BPS Guide to Pharmacology. Mode of access: https://www.guidetopharmacology.org (access from May 29, 2023)
33. Donahue TJ, Hillhouse TM, Webster KA, Young R, De Oliveira EO, Porter JH (2017) Discriminative stimulus properties of the atypical antipsychotic amisulpride: comparison to its isomers and to other benzamide derivatives, antipsychotic, antidepressant, and antianxiety drugs in C57BL/6 mice. Psychopharmacology (Berl) 234(23–24): 3507–3520. https://doi.org/10.1007/s00213-017-4738-y
34. Meltzer HY, Bobo WV (2012) Antipsychotic and anticholinergic drugs. In: M Gelder (ed) New Oxford Textbook of Psychiatry 2 ed. Oxford Acad, Oxford.

Translated by A. Dyomina