ISSN 0022-0930, Journal of Evolutionary Biochemistry and Physiology, 2022, Vol. 58, No. 4, pp. 1130–1141. © Pleiades Publishing, Ltd., 2022.
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EXPERIMENTAL PAPERS

Use of Naïve Bayes Classifier to Assess the Effects of Antipsychotic Agents on Brain Electrical Activity Parameters in Rats

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Received May 23, 2022

Revised June 16, 2022

Accepted June 20, 2022

Abstract—Research and development of novel methods to determine the effects of antipsychotic agents is an important challenge for experimental biomedicine. Although behavioural tests, the ones most commonly used for pharmacological screening, are quite efficient for the evaluation of drug effects on animal anxiety and locomotion, they hardly allow to detect antipsychotic activity. Pharmacoelectroencephalography (pharmaco-EEG), which is based on the principle of different psychoactive agents producing distinct changes in brain electrical activity, could represent a viable alternative approach to that task. The rapid evolution of machine learning techniques has opened new possibilities for using pharmaco-EEG data for the purposes of classification and prediction. This work describes an experimental approach to the assessment of specific activity and pharmacological profiling of antipsychotic agents using naïve Bayes classifier, a simple probabilistic classifier widely employed in biomedical research. The experiments were conducted in white outbred male rats with chronically implanted electrocorticographic electrodes. To serve as the training set, a library was assembled containing electrocorticograms (ECoG) following the administration of antipsychotic agents: chlorpromazine, haloperidol, droperidol, tiapride, and sulpiride. For each sample, ECoG parameters before and after drug administration were calculated, and a total of 132 amplitude and spectral signal parameters were taken into analysis. Principal component analysis was used to reduce dimensionality. Using naïve bayes classifier, we were able to detect and qualify distinct effects of antipsychotic agents on brain electrical activity parameters in rats, allowing them to be differentiated from phenazepam, a benzodiazepine tranquilizer with sedative properties. Moreover, this approach proved effective to distinguish among the antipsychotics as well as between them and other agents with similar receptor binding affinity profiles, e.g., the tricyclic antidepressant amitriptyline. Thus, the method we propose can be used to discern between antipsychotic and sedative effects of drugs as well as to compare the effects across different antipsychotic agents.

DOI: 10.1134/S0022093022040160

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

Antipsychotics are a group of drugs widely used in psychopharmacology, mainly for the treatment of schizophrenia and affective disorders. With the appearance of new representatives of this group, many of them have been shown to have additional positive effects in bipolar (olanzapine, risperidone, quetiapine) and unipolar depression (aripiprazole, olanzapine, etc.), as well as in anxiety disorders (quetiapine) [1]. It is generally accepted that the main pharmacological target of antipsychotic drugs are dopamine D_2-receptors, but a modern approach to classification suggests distinguishing at least 5 groups, depending on the mechanisms of action [2], and pharmacological targets may include not only dopamine receptors, but also other mediator systems, including serotonergic, glutamatergic, and γ-aminobutyric acid (GABA) systems, as well as trace amine associated receptors (TAARs) and neuropeptides [3].

Due to the great variety of molecular mechanisms of action of various antipsychotic agents, there is a need for effective methods of differentiating the effects of representatives of this group at the stage of experimental research. Traditionally, a battery of behavioral tests can be used to assess the level of anxiety and exploratory activity of small laboratory animals (e.g., "Open field", "Elevated plus maze", etc.). The next step is usually experiments using models of psychotic disorders in mice and rats (destruction of ventral hippocampal regions, pharmacological and genetic models) [4] and specific behavioral tests (prepulse inhibition, three-chamber sociability test, etc.) [5]. When several behavioral tests are performed at once, comprehensive data on the effects of a particular drug on schizophrenia symptoms in laboratory animals can be obtained. Nevertheless, this approach may require a large amount of time, material costs, and compliance with rather strict testing conditions (sequence of tests, intervals between tests, etc.). In addition, behavioral tests are not always sensitive enough to detect the effects of psychoactive drugs.

Pharmacoencephalography (pharmaco-EEG) may be a promising approach for detecting differences in the effects of antipsychotic drugs. Despite the fact that the first such studies were conducted in the 1960s and 1970s [6, 7], this experimental approach has never become a standard for pharmacological screening of new psychoactive molecules. This was largely due to the complexity of the data obtained (a large number of indicators, often difficult to interpret), due to which researchers could not use them to compare the profiles of the pharmacological activity of the studied drugs. With the advent of modern methods of computer data analysis, interest in the use of pharmaco-EEG in biomedical research has now increased again [8–11].

The possibility of using machine learning methods to predict the pharmacological activity of a drug based on the data on its effects on the parameters of bioelectrical activity in laboratory animals attracts particular attention. Currently, such approaches are actively used for the targeted synthesis of new pharmacologically active compounds [12]. Despite the great variety of machine learning methods, the naïve Bayes classifier (NBC), being one of the most basic ones, is widely used in medical and biomedical research. The NBC is a simple probabilistic classifier in which each parameter of the classified data is considered independent of the other characteristics. It is widely used in medical practice, for example, to predict the resistance of cancer patients to chemotherapy [13], to diagnose diabetes mellitus [14], or to assess the risk of drug-induced liver damage [15]. Of course, the possibilities of application of this algorithm are not limited to the above examples, and every year there are more and more works where authors successfully use this approach in classification and prediction tasks [16].

Based on the above, the purpose of this work was to evaluate the possibility of using the NBC to detect and differentiate the effects of antipsychotic drugs on electrocorticogram (ECoG) parameters in rats.

MATERIALS AND METHODS

The study was performed according to the principles of the Basel Declaration, the Order of the Ministry of Health of the Russian Federation from 01.04.16 no. 199n "On approval of the rules of good laboratory practice" and the recommendations of the Bioethical Commission of the Federal State Budgetary Educational Institution of Higher Professional Education SPCFU of the Ministry of Health of Russia. Experiments were performed on 33 male Wistar rats weighing 250–300 g obtained from Rappolovo Laboratory Animal Supplier (Leningrad Region, Russia). Rats were kept under standard vivarium conditions on the standard chow, with *ad libitum* access to water. All experimental and control animals were taken from the same batch and quarantined for 14 days.

Corticographic electrodes were made from 0.5 mm diameter nichrome wire (for the recording and reference electrodes) and 0.16 mm diameter for the grounding electrode. Isolation was performed with heat-shrink tubing (1.5/0.5 mm); the length of the recording (uninsulated) part was ≈1 mm. All electrodes were connected in a 2.54 mm pitch BLS-8 header connector (Connfly Electronic Co. Ltd., PRC).

The procedures of electrode implantation and postoperative care of the animals were detailed in a previously published paper [17]. Tiletamine/zolazepam 50 (Zoletil®, Virbac, France; 10 mg/kg, intramuscularly) was used to anesthetize the animals. Electrodes FP1 and FP2 were placed in the primary motor cortex (AP = 0.0, ML = 2.5, DV = 1.0), C3 and C4 in the primary somatosensory cortex over the hippocampus (AP = –4.0, ML = 2.5, DV = 1.0), O1 and O2 in the secondary visual cortex (AP = –7.0, ML = 2.5, DV = 1.0). The reference electrode was placed in the nasal bone, and the grounding electrode was placed under the skin in the neck region.

ECoG recording in animals was performed not earlier than 7 days after the operation using an 8-channel Neuron-Spectrum-1 EEG system (Neurosoft, Russia) at a 0.5–35 Hz bandwidth and a 500 Hz sampling rate. The signal was recorded simultaneously with video recording of behavior in the home cage conditions under artificial light. The recording duration was 2 h and included 30 min of background activity (before drug or physiological solution injection) and 1.5 h after the injection. Two 60-second recording fragments were selected for further analysis: immediately before the injection and 20 min after. During the selected ECoG fragments, the animals were in a calm awake state, with no locomotor or exploratory activity or grooming or scratching [18].

As typical antipsychotic drugs we chose chlorpromazine, haloperidol and droperidol, as atypical were chosen tiapride and sulpiride. In addition, as reference drugs we made ECoG records of the tricyclic antidepressant amitriptyline, acetylcholinesterase inhibitor galantamine and benzodiazepine tranquilizer phenazepam. The choice of amitriptyline as a comparison drug was due to its action on many of the pharmacological targets (M-choline, H_1-histamine, and α_1-adrenoreceptors) that are the targets of chlorpromazine. It was of interest to what extent the proposed approach would detect this similarity.

It is also known that typical neuroleptics can cause extrapyramidal disturbances by increasing the tone of the cholinergic innervation in the brain [19]. In the present work it was assumed that the pharmaco-EEG method might be sensitive to early manifestations of this side effect, and in view of this for chlorpromazine, haloperidol and droperidol a high probability of similarity with galantamine as an agent increasing the tone of cholinergic innervation was expected. Phenazepam was chosen as an agent with a pronounced sedative effect, the effect of which on ECoG parameters should differ from that of typical and atypical antipsychotics.

The test drugs were injected intraperitoneally, if necessary, pre-dissolved in normal saline to the desired concentration. The dose of haloperidol (OOO Velpharm, Russia) and droperidol (FSUE Moscow Endocrine Plant, Russia) was 0.3 mg/kg, chlorpromazine (Valenta Farm JSC, Russia)—10 mg/kg, amitriptyline (Moscow Endocrine Plant JSC, Russia), tiapride (Organika JSC, Russia) and sulpiride (Organika JSC, Russia)—30 mg/kg, galantamine (Sopharma JSC, Bulgaria) and phenazepam (Valenta Farm JSC, Russia)—1 mg/kg [20–22]. As a control, 0.5 mL of normal saline was administered.

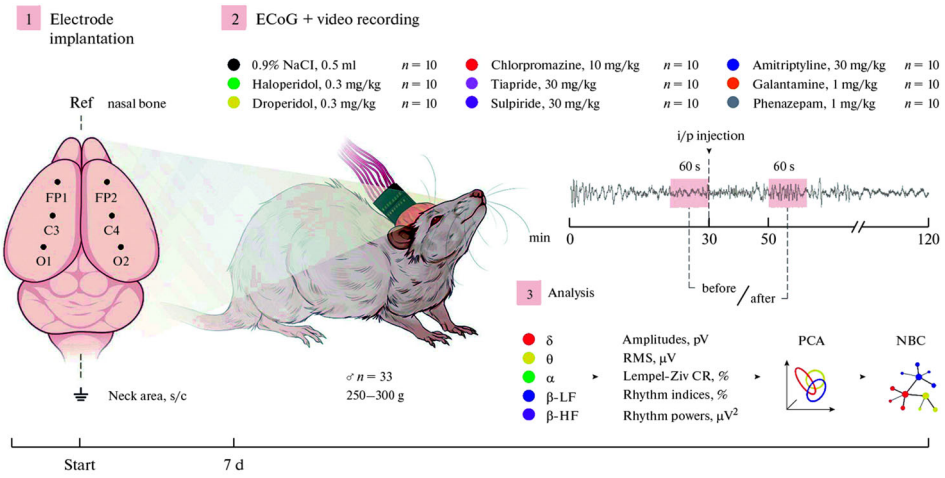


Fig. 1. Stages of the experiment. (1) Electrocorticographic electrode implantation in the primary motor cortex area (FP1 and FP2), secondary sensorimotor cortex area above the hippocampusC3 and C4), and secondary visual cortex area (O1 and O2). (2) Electrocorticogram acquisition in home cage conditions with synchronous video registration of behaviour. (3) Analysis of selected electrocorticogram fragments. Ref—Reference electrode, s/c—subcutaneously, i/p—intraperitoneally, ECoG—electrocorticogram, RMS—root mean square, CR—compression ratio, LF—low-frequency, HF—high-frequency, PCA—principal component analysis, NBC—naïve Bayes classifier.

At least 10 recordings were made for each drug in different animals (n = 10 for each group). A new drug was administered no earlier than 3 days after the previous recording to exclude interactions and residual effects. The frequency of testing one or another rat was determined by the integrity of the connectors and the ground electrode, as well as by the general condition of the animal. In case of signs of infectious disease, bicillin-3 (Sintez, Russia; 5000 IU/kg, intramuscularly) was reinjected and the next test was performed not earlier than one week after the injection.

The obtained recordings were analyzed using the Neuron-Spectrum.NETω program (Neurosoft LLC, Russia). For all 6 leads (FP1, FP2, C3, C4, O1, and O2) we performed amplitude and spectral analysis with calculation of a total of 132 parameters, including mean and maximum signal amplitudes, standard deviation and Lempel–Ziv compression ratio, mean amplitudes of wave rhythms, indices, and mean power of rhythms. 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 isolated from the signal. The data were expressed as ratios of the parameter values before drug administration to the values of the corresponding parameters after injection (from 0 to 1) (Fig. 1).

The data were processed and analyzed using the add-on for MS Excel XLSTAT 2016.02.28451. Data dimensionality reduction was performed using the principal components analysis (PCA), a technology of multivariate statistical analysis used to reduce the dimensionality of the parameter space with minimal loss of useful information. The essence of the method is an orthogonal linear transformation that maps the data from the original parameter space into a new space of smaller dimensionality. The first axis of the new coordinate system is constructed in such a way that the dispersion of data along it would be maximum. The second axis is orthogonal to the first one, so that the dispersion of data along it would be the maximum of the remaining possible, etc. The first axis is called the first principal component, the second the second, etc. [23]. Based on the calculated values of the principal components for each entry from the chlorpromazine, haloperidol, droperidol, tiapride and sulpiride groups, pharmacological activity was predicted using NBC (similarity to one or another training set was calculated). As a training set, data from all records obtained except for the group of the drug for which the prediction was made were used. The numerical data shown in the figures are presented as mean ± standard error of the mean (SEM).

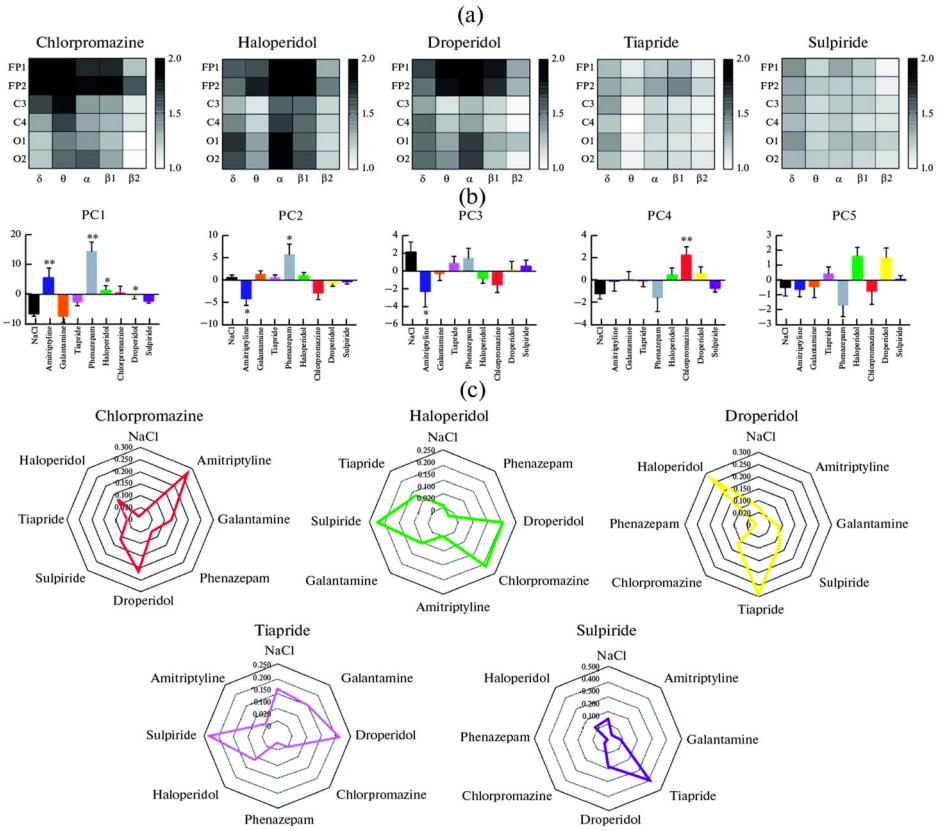
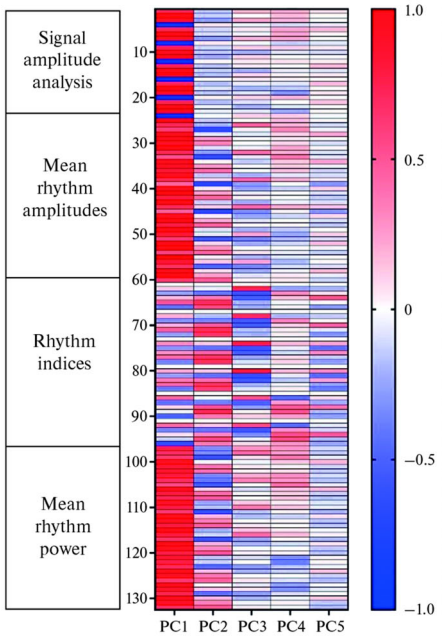


Fig. 2. Electrocorticogram parameter analysis results. (a) Heatmaps of averaged (n = 10 for each group) mean power values for δ, θ, α, and β rhythms in leads FP1, FP2, C3, C4, O1, and O2 following the administration of chlorpromazine, haloperidol, droperidol, tiapride, and sulpiride. Data are presented as ratios of mean rhythm power values at 20 min after drug administration to corresponding values before drug administration (at 30 min of baseline recording). (b) Mean values of the five principal components across all experimental groups. (c) Radar charts of mean match probabilities for the pharmacological effects of chlorpromazine, haloperidol, droperidol, tiapride, and sulpiride, and those of the drugs from the training set, as predicted by the naïve Bayes classifier.

RESULTS

Administration of antipsychotic drugs to rats caused changes in the amplitude and spectral characteristics of the ECoG (Fig. 2a). The most pronounced effects were noted for chlorpromazine, haloperidol, and droperidol. When chlorpromazine was administered, there was an increase in the average power of δ-, θ-, α-, and β-LF rhythms mainly in the frontal cortex (electrodes FP1 and FP2). Haloperidol increased the average power of predominantly α- and β-LF-rhythms in the FP1 and FP1 leads, as well as α-rhythms in the occipital area. Droperidol had similar effects, while additionally activating θ-activity in the frontal cortex. Tiapride and sulpiride, like the previous drugs, increased the average power in δ-, θ-, α- and β-bands, but their effects, unlike chlorpromazine, haloperidol and droperidol, were not localized in any particular cortical area and were less pronounced.

When analyzing the PCA data, it was obtained that the first five components (PC1–PC5) describes 84.8% of the total variance. They were used for further calculations. For each of the analyzed ECoG parameters, the corresponding factor loadings were obtained, allowing us to estimate the contribution of these parameters to the formation of a particular principal component (Fig. 3). The PC1 component, which describes 55.3% of the data dispersion, was formed by such amplitude characteristics of the signal as maximum and mean amplitudes, standard deviation, Lempel–Ziv compression ratio, and mean amplitudes and powers of δ-, θ-, α- and β-rhythms. It is important to note that all the listed ECoG parameters influenced the value



#	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	Lempei-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	Lempei-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	Lempei-Ziv	C3	56	Delta amp	O2	100	A^Jhamp	FP1
13	Max amp	C4	57	Theta amp	O2	101	Beta-LF mp	FP1
14	Min »np	C4	58	Alpha amp	O2	102	Beta-HF mp	FP1
15	RMS	C4	59	Beta-LF amp	O2	103	Band pass mp	FP2
16	Lempei-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	Lempei-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 anp	O2	66	Beta-HF ind	FP1	110	Delta mp	C3
23	RMS	O2	67	Band pass ind	FP2	111	Theta mp	C3
24	Lempei-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 passmp	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	A|>hamp	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	O4	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 representing the weights of each of the 132 parameters analyzed for the principal components PC, PC2, PC3, PC4, and PC5, to be further analyzed using 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).

of PC1 regardless of the recording lead. The PC2 component accounted for 13.4% of the variance, and its values were most influenced by the indices and mean power of β-LF- and HF-rhythms in all signal registration areas. The PC3 component (8.4% of the variance) was composed of the ratio of δ-, θ-, and α-rhythm indices, regardless of signal localization. PC4 (4.2%) was formed by the mean amplitude of θ-rhythms in the C3 and C4 leads (primary sensorimotor cortex area above the hippocampus), as well as the α-rhythm index in all leads. The PC5 component described 3.5% of the variance and was determined by the values of β-LF rhythm indices in the occipital electrode regions O1 and O2.

Thus, the ECoG parameter values (132 indices) were recalculated for each record to the values of the main components PC1–PC5 (Table 1). It was noted that the studied drugs had a pronounced effect on the values of PC1–PC5 indices, and their specific and multidirectional effect was revealed (Fig. 2b). Administration of amitriptyline, phenazepam, haloperidol and droperidol statistically significantly ($p < 0.01$ for amitriptyline and phenazepam, $p < 0.05$ for haloperidol and droperidol) increased the PC1 component compared to controls. The PC2 value decreased with amitriptyline administration ($p < 0.05$) and, in contrast, increased in animals receiving phenazepam ($p < 0.05$). Rats treated with amitriptyline also showed a decrease in PC3 component compared to controls ($p < 0.05$). The PC4 component increased statistically significantly ($p < 0.01$) when chlorpromazine was administered. None of the test drugs had a significant effect on the PC5 component, but there was a tendency for it to increase in the haloperidol and droperidol groups, and to decrease in the phenazepam group.

The next step was to classify the electrocorticograms obtained based on their principal component values using the NBC. For each record analyzed, we calculated the probability ratios for the coincidence of effects on the ECoG with the effects of other groups of drugs used in the training set (Table 2). The obtained values were averaged for each group, from which conclusions could be drawn about the similarity of the effects of certain psychotropic drugs (Fig. 2c). For example, chlorpromazine was shown to have the most similar effects to amitriptyline and droperidol (probability fractions of 0.281 and 0.216, respectively). The effects of haloperidol resembled those of droperidol (0.206), chlorpromazine (0.210) and sulpiride (0.220) to a greater extent. For halo-

Table 1. An example of calculated principal component PC1–PC5 values for 10 electrocorticograms acquired following NaCl administration

Electrocorticogram	Drugs				
	NaCl	Amitriptyline	Galantamine	Tiapride	Droperidol
1	0.290	0.024	0.059	0.421	0.172
2	0.037	0.042	0.101	0.396	0.228
3	0.176	0.009	0.120	0.462	0.102
4	0.017	0.147	0.018	0.215	0.569
5	0.084	0.005	0.061	0.641	0.120
6	0.221	0.003	0.067	0.569	0.079
7	0.255	0.014	0.108	0.448	0.088
8	0.008	0.006	0.072	0.353	0.124
9	0.047	0.018	0.080	0.446	0.213
10	0.188	0.128	0.181	0.100	0.152

Table 2. An example of calculated match probabilities for the effects of sulpiride in 10 animals, and those of the other drugs

Electro-cortico-gram	Drugs							
	NaCl	Amitripty-line	Galantam-ine	Tiapride	Droperidol	Chlor-promazine	Phenaze-pam	Haloperidol
1	0.290	0.024	0.059	0.421	0.172	0.008	0.002	0.023
2	0.037	0.042	0.101	0.396	0.228	0.017	0.006	0.173
3	0.176	0.009	0.120	0.462	0.102	0.021	0.000	0.109
4	0.017	0.147	0.018	0.215	0.569	0.018	0.012	0.004
5	0.084	0.005	0.061	0.641	0.120	0.008	0.001	0.080
6	0.221	0.003	0.067	0.569	0.079	0.005	0.000	0.055
7	0.255	0.014	0.108	0.448	0.088	0.007	0.003	0.076
8	0.008	0.006	0.072	0.353	0.124	0.020	0.001	0.416
9	0.047	0.018	0.080	0.446	0.213	0.023	0.001	0.172
10	0.188	0.128	0.181	0.100	0.152	0.212	0.001	0.037

peridol, similarity with droperidol (0.268) and tiapride (0.288) was shown, and for tiapride with sulpiride (0.231) and droperidol (0.214). The most selective prediction was for sulpiride, with a probability fraction of similarity to tiapride of 0.405. None of the antipsychotics showed a high probability of similarity to NaCl or phenazepam. The NBC showed moderate similarity of the effects of the test agents with galantamine, with tiapride showing the greatest similarity (0.147) and droperidol and sulpiride showing the least similarity (0.860 and 0.870).

DISCUSSION

The present work shows that the pharmaco-

Table 3. Receptor inhibition constant values (Ki, nM), representing binding affinities of the antipsychotics from this study towards different receptors [25–27]

Receptor	Drugs				
	Chlorpromazine	Haloperidol	Droperidol	Sulpiride	Tiapride
Dopamine receptors					
D_1	6.3	83	880	N/A	N/A
D_2	11	2.0	0.25	8.2	226
D_3	9.7	4.0	N/A	7.9	324
D_4	56	48	0.84	54	14
D_5	N/A	147	N/A	N/A	N/A
Serotonin receptors					
5-HT_{1A}	840	1200	N/A	N/A	N/A
5-HT_{2A}	N/A	70	4.6	>10000	N/A
5-HT_{2C}	N/A	5000	N/A	N/A	N/A
Other					
α_1-AR	1.4	12	N/A	N/A	N/A
H_1-HR	25	3000	N/A	N/A	N/A
M_1-AChR	1.5	>10000	N/A	N/A	N/A

AR—Adrenergic receptor, HR—histamine receptor, AChR—acetylcholine receptor, N/A—data not available.

ECoG method in rats in combination with PCA and NBC can be used to compare the profiles of antipsychotic agents and to distinguish their effects from agents with antidepressant and sedative effects (using amitriptyline and phenazepam as examples). For all five studied antipsychotics, similarities with the most similar drugs in terms of pharmacological action were shown. For example, the effects of chlorpromazine on the amplitude and spectral characteristics of the ECoG were closest to those of droperidol and haloperidol, which is understandable, since all drugs have a strong antipsychotic effect associated with a pronounced or moderate blockade of D_2 receptors [2]. The similarity between chlorpromazine and amitriptyline is also not unexpected, because despite the fact that the drugs represent different pharmacological groups, both exhibit antagonism to M-choline, α_1-adreno- and H_1-histamine receptors [2, 24].

Similarly, the nature of haloperidol action was similar to that of droperidol and chlorpromazine. Nevertheless, the high probability of coincidence of its effects with sulpiride was unexpected. On the one hand, both drugs are antipsychotics, but for haloperidol the probability of coincidence with another atypical neuroleptic, tiapride, was low. It is noteworthy that droperidol, unlike haloperidol, was shown to have a high degree of similarity to tiapride. Thus, a tentative line can be drawn between haloperidol, which is similar to chlorpromazine and sulpiride, and droperidol, which is similar in its effects to tiapride. Based on data from in vitro studies, we can assume that the similarity of the latter two is due to their greatest affinity for D4 receptors (Table 3). The pronounced similarity between sulpiride and tiapride most probably reflects the fact that both drugs are similar in chemical structure (they are substituted benzamides). It should also be taken into account

that the EEG picture of the drugs' action may be affected by their different selectivity toward receptors of the same type, but located in different areas (nigrostriatal, mesocortical, or mesolimbic).

One of the working hypotheses of the present study was that the approach being implemented might be suitable for detecting early signs of possible extrapyramidal disturbances in animals resulting from the administration of so-called typical antipsychotics. Since the development of extrapyramidal disorders such as bradykinesia, muscle rigidity, and tremor when using typical neuroleptics is largely associated with excessive cholinergic innervation tone in the brain [19], it has been suggested that such changes in neurotransmitter systems can be detected by ECoG recording at an early stage. That is why galantamine, an acetylcholinesterase inhibitor with a pronounced cholinergic effect, was chosen as one of the reference agents. Despite the fact that in this work, some similarity with galantamine was shown for all five antipsychotics (almost always higher than with NaCl or phenazepam), the result cannot be considered positive, because the most "cholinotropic" was tiapride, which in clinical practice does not often cause extrapyramidal disorders [28]. Nevertheless, the idea of searching for early predictors of possible extrapyramidal disorders using pharmaco-EEG remains attractive and deserves attention in further studies.

The doses of drugs having a pronounced effect on the behavior or ECoG parameters of animals given in previously published works were used in this work. Undoubtedly, the question of dose selection is one of the key ones for solving classification and prediction problems, because the calculated probabilities of assigning a drug to one or another group can change dramatically when the dose is repeatedly increased or decreased by orders of magnitude. For example, for antipsychotics, sedative and anticholinergic effects arise with increasing doses, which will certainly affect the recorded bioelectrical activity of the brain [29]. In this connection, for further development of the proposed method as a tool for pharmacological screening, it is necessary to supplement the training set with recordings of the effects of known drugs in several dose ranges.

The idea of using pharmaco-EEG in laboratory animals is not a fundamentally new approach in biomedical research. This method has been repeatedly used by different scientific groups to characterize the functional effects of potential or already known psychoactive compounds [30–33], to identify possible side effects (e.g., somnogenic) [34] or toxic effects (epileptogenic activity, etc.) [35].

The research of the scientific group of W. Dimpfel, the first results of which were published in the mid-80s and continue to be published today, deserves special attention. In a 1984 paper [36], the authors applied discriminant analysis to distinguish the effects of sulpiride, clozapine and haloperidol based on a comparison with the effects of amphetamine, diazepam, imipramine and chlorpromazine. As input data we used the ratios before and after drug administration of the absolute powers of δ-, θ-, α-, and β-rhythm signals recorded bilaterally in the projection areas of the sensorimotor cortex, striatum, and reticular formation. Subsequent works were devoted to recording the effects of different groups of drugs, which subsequently allowed the authors to collect a library of so-called electropharmacograms [37], including neuroleptics, opioid and non-opioid analgesics, antidepressants, psychostimulants, sedatives and anticonvulsants, and even hallucinogens (LSD, MK 801, etc.). From 2009 to the present, this library has been actively used to study the effects of herbal extracts. For example, the antidepressant effects of the flavonoids rutin and quercetin [20] and several other compounds were predicted, and a variant of the pharmacological classification of plant extracts based on their electropharmacograms was proposed [38].

A similar approach was developed by the Dutch researchers Krijzer et al. in 1993. [30]. Registration of bioelectrical activity of the brain was performed from the frontal and parietal cortical areas (a differential signal was obtained), 256 spectral band power ratios from 0.36 to 100 Hz before and 20 and 45 min after drug administration were used as input data for further analysis. Analysis of variance, *t*-test, and subsequent normalization by degrees of freedom transformed the primary analysis values into so-called n-profiles. Further discriminant analysis allowed us to compare the n-profiles of drugs from different groups and to dis-

tinguish between the effects of antidepressants, neuroleptics, anxiolytics, and psychostimulants. The proposed method made it possible to predict the antidepressant and anxiolytic effects of E-10-hydroxynortriptyline, the active metabolite of nortriptyline, which confirmed previous clinical observations in depressed patients [31]. Despite the positive results, this approach has not been developed, and the above study is the last published work that used n-profiles to predict pharmacological activity.

FGBUN Scientific Center for Biomedical Technologies FMBA of Russia (Moscow region) proposed another prediction method in which the cat was chosen as the test system [39, 40]. The bioelectrical activity of the brain was recorded using depth electrodes implanted in the cingulate gyrus, hypothalamus, caudate nucleus, and other structures. Assessment of the pharmacological activity profile of the tested psychotropic drugs was based on comparing the normalized spectral power values with those of the reference drugs. Using this method, it was shown that leucineenkephalin leutragin has similar effects to the original synthetic analogue of an adrenocorticotropic hormone fragment (ACTH4-10), which, according to the authors, reflects mechanisms of GABAergic modulation of the hippocampus and prefrontal cortex [41].

Thus, the results of the above-mentioned works indicate that the pharmaco-EEG method in laboratory animals can be used to detect and determine the effects of drugs affecting the functions of the central nervous system. The example of studies by Dimpfel et al. shows that this experimental approach has not remained a rudiment of neurobiological research, but can be successfully used to solve research problems at present. Based on the results of this study and comparison with the works of other authors, we can conclude that different mathematical approaches can be used for solving classification and prediction problems. Analysis of the data of bioelectrical activity of the brain allows calculating tens and even hundreds of amplitude and spectral characteristics of signals; however, for successful application of various classifiers, competent approaches of dimensionality reduction or identification of the indicators most sensitive to pharmacological effects are required. Especially important is that with the emergence of new machine learning methods, the data obtained do not lose their relevance and can provide fertile ground for research work not only by neuropharmacologists, but also by specialists from the fields of mathematics and information technology.

AUTHORS' CONTRIBUTION

Idea of work and planning of (Y.I.S., S.V.O.) conducting experiments and data processing (Y.I.S., D.D.S., M.M.P., V.A.P., R.D.I., A.A.K.), preparing illustrations (Y.I.S., D.D.S., V.A.P.), preparing and editing the manuscript (Y.I.S., V.A.P., S.V.O.).

FUNDING

The work was performed using the equipment of the Analytical Center of the Russian Ministry of Health under Agreement No. 075-15-2021-685 of July 26, 2021, funded by the Russian Ministry of Education and Science. This work was performed within project ID: 93022798 (for Y.S.) of the St. Petersburg State University, St. Petersburg, Russia, and supported by the Russian Science Foundation grant 22-15-00092 (for developing of experimental setup).

CONFLICT OF INTEREST

The authors declare that they have neither evident nor potential conflict of interest related to the publication of this article.

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Translated by A. Dyomina