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IMPACT OF VORTIOXETINE WITH ANTIEPILEPTIC DRUGS COMBINED ADMINISTRATION ON NON- CONVULSIVE BEHAVIOUR IN KINDLED RATS

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Abstract

Epilepsy is a prolonged, progressively advancing nervous and psychiatric disorder of various etiologies, characterized by paroxysmal and relatively persistent psychological disturbances. A significant burden of comorbid pathology is attributed to psychiatric disorders. Depressive disorders are among the most common behavioral comorbidities in patients with epilepsy, often remaining undiagnosed and untreated. The approach to treating depression in epilepsy patients is currently underdeveloped. To fully understand the prospects of the experimental framework for minimizing and/or eliminating depressive behavioral manifestations during the interictal period under conditions of chronic epileptogenesis, we conducted a series of studies to determine the effectiveness of comprehensive correction of non-seizure behavior in kindling animals. The aim of this study is to delineate alterations in the expression of non-seizure behavior in rats due to the combined administration of Vortioxetine with antiepileptic drugs in the dynamic context of chronic kindling-induced seizure activity. It has been established that in rats during the development of Picrotoxin-induced chronic epileptic

syndrome, disruptions in late-tonic, emotional, and swimming types of behavior are observed, which are manifestations of non-seizure behavior. It has been proven that the combined administration of Valproic acid and Vortioxetine results in successful correction of the tested forms of behavior. Less effectiveness is observed with vortioxetine alone, and the least effectiveness is noted in a smaller number of investigated behavior types after valproic acid administration. The data demonstrate balancing systemic-antisynergistic relationships, the imbalance of which during the development of picrotoxin-induced chronic epileptic syndrome allows the development of non-seizure behavior forms during interictal periods. The obtained data and their analysis provide experimental groundwork for the appropriateness of clinically testing the combined administration of Vortioxetine with Valproic acid as part of complex therapy in epilepsy patients with the presence of a depressive behavioral component.

Key words: epilepsy; kindling; depression; non-convulsive behaviour; Vortioxetine; Valproic acid; Phenobarbital; Diphenylhydantoin; pathogenetic correction

Epilepsy is a chronic and progressive neurological and psychiatric disorder with diverse etiologies, characterized by paroxysmal and relatively persistent psychological disturbances [1, 9, 31, 33]. The prevalence of epilepsy varies between 49 and 100 cases per 100,000 population across different countries [4], showing even higher rates in Eastern European and developing nations [28]. The frequency of chronic epilepsy is estimated to range from 1 in 200 patients [12] to 4-10 per 1000 patients [15]. Annually, 50-70 cases per 100,000 population are reported [28].

While involuntary recurrent seizures are a defining feature of this chronic condition and can lead to fatal outcomes for patients, the most substantial negative impact associated with epilepsy emanates from comorbidities that significantly erode the quality of life and contribute to disability [14]. The clinical spectrum of this polyetiologic disorder encompasses a wide array of somatic, physical, neurobiological, neuropsychological, pathopsychological, and social manifestations [13].

A significant burden of comorbid pathology is linked to psychiatric disorders [10, 11, 13, 16]. The significance of these concerns has been underscored by the National Institute of Health, which has prioritized the investigation of comorbid psychiatric disorders in epilepsy patients [22].

In this context, evidence has surfaced indicating that depressive disorders rank among the most prevalent behavioral comorbidities in epilepsy patients, frequently evading diagnosis and treatment [17, 24]. Clinical observations highlight that depressive disorders are

particularly pronounced in patients newly diagnosed with epilepsy during the initial year of their affliction [16]. The prevalence of depression in epilepsy patients ranges from 11.2% to 60.0% [10, 22], significantly surpassing that in the general population (2-4%).

Experts contend that frequent and severe episodes of psychological stress constitute risk factors for the onset of seizures and contribute to the development of epilepsy [9, 18]. This assertion finds support in the exacerbation of seizure severity and frequency following stress in epilepsy patients, as well as the potential attenuation of seizure reactions through psychiatric anti-stress interventions [21, 23]. Empirical evidence has indicated that a history of depression amplifies the risk of epileptic seizures by 3-7 times [6, 23], complicates prognostication of the underlying malady [25], and heightens the likelihood of suicide attempts [20, 34].

The approach to treating depression in epilepsy patients remains underdeveloped. Isolated studies are dedicated to the utilization of antidepressants in patients afflicted by epilepsy and depression. This is attributed to the intricacy of treating such individuals, taking into consideration factors like the interplay between antiepileptic drugs and antidepressants. Antidepressants do not reduce the seizure threshold unless administered in very high doses [14]. Furthermore, the administration of therapeutic doses of antidepressants such as Chlorimipramine, Mirtazapine, and Bupropion is associated with seizure occurrence [14]. Nonetheless, experimental and clinical data corroborate the antiepileptic efficacy of selective serotonin reuptake inhibitors [19]

We have already undertaken a series of experimental studies and clinical observations aimed at rectifying mnemonic impairments in kindling rats and patients suffering from post-traumatic epilepsy under the context of combined administration of antiseizure drugs and the multimodal antidepressant Vortioxetine (Brintellix) [4, 26]. To comprehensively grasp the potential of the experimental framework in minimizing and/or mitigating depressive behavioral manifestations during the interictal period amidst chronic epileptogenesis, a series of studies were conducted to evaluate the effectiveness of a comprehensive correction strategy for non-seizure behavior in kindling animals.

The aim of the work is to delineate alterations in the expression of non-seizure behavior in rats due to the combined administration of Vortioxetine with antiepileptic drugs in the dynamic context of chronic kindling-induced seizure activity.

Materials and methods

Experiments were conducted under controlled conditions on 86 male Wistar rats weighing 180-250 g, which were housed in vivarium conditions. Ethical considerations, as outlined by the 'General Ethical Principles in Animal Experiments' from the Fifth National

Bioethics Congress (Kyiv, 2013), European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985), and State Expert Centre guidelines of the Ministry of Health of Ukraine on 'Preclinical Drug Development' (2001), were strictly adhered to throughout animal care, management, and manipulations.

To induce chronic seizure syndrome, a chemical kindling model was employed, involving the 24-hour intraperitoneal administration of Picrotoxin (PTX, Sigma-Aldrich, Germany; prepared as a 0.5% solution) dissolved in 0.9% physiological saline solution, at a subthreshold dose ranging from 0.9 to 1.1 mg/kg [1].

The rats were randomized as following: 1) Control group (n=9), receiving 0.9% physiological saline intraperitoneal (i.p.) administration; 2) Kindled rats in one stage of kindling (n=11); 3) Kindled rats receiving Valproic acid (VPA, Sigma-Aldrich, Germany; 100 mg/kg, i.p.); 4) Kindled rats receiving Phenytoin (DPH, Sigma-Aldrich, Germany; 100 mg/kg, i.p.); 5) Kindled rats receiving Phenobarbital (PB, Sigma-Aldrich, Germany; 5.0 mg/kg, i.p.); 6) Kindled rats receiving Vortioxetin (VT, Lundbeck, Denmark; 5.0 mg/kg, subcutaneously); 7) Kindled rats receiving both VPA and VT; 8) Kindled rats receiving both DPH and VT; 9) Kindled rats receiving both PB and VT; 10) Kindled rats receiving an opioid receptor blocker Naloxone (NAL, Dupont, USA; 1 mg/kg, i.p.); 11) Kindled rats receiving a non-competitive antagonist of glutamate/aspartate receptors Ketamine (KET, Callipsol, Gedeon Richter, Hungary; 5 mg/kg, i.p.); 12) Kindled rats receiving an M-cholinoblocker Atropine (ATR, Sigma-Aldrich, Germany; 1 mg/kg, i.p.).

The mentioned substances were dissolved in a 5% solution of Methylcellulose (Methocel, Serva, Germany) and administered 60 minutes before PTX injection.

The exploration of non-seizure behavior in rats was conducted across distinct stages within three time intervals: after the 18^{th} PTX injection (stage of developed chronic seizure syndrome), following the 24^{th} PTX injection (completion of pharmacological kindling), and after a 14-day interval post the completion of kindling formation (38 days into the study, post-kindling stage) [30]. During these intervals, the rats' expressions of posture behavior [24, 35], emotional reactions in the test of aggressive-defensive behavior [3, 27], and swimming behavior [2, 37] were meticulously assessed.

The resulting data underwent rigorous statistical analysis employing both parametric and non-parametric methods. To ascertain interval values' likelihood (pain reaction intensity, emotional behavior expression, passive-adaptive act count, and the ability to transition to active-adaptive swimming behavior), a parametric ANOVA test was employed, complemented by the

Neuman-Keuls test where appropriate. For nominal values (frequency of animals with specific manifestations of late-tonic behavior, variability metrics, and maximum variability), a non-parametric Kruskal-Wallis test was employed. Statistical significance was established at $p<0.05$.

Results

1. Pharmacological Correction of Posture Behavior

Following the 18th PTX injection during the progression of kindling, rats exhibited posture behavior characterized by the splaying of forelimbs when placed on a horizontal surface (observed in 5 out of 9 rats; Table 1). When placed on their side, 8 out of 9 animals remained in an uncomfortable position for over 1 min. Exophthalmos was noted in 8 out of 9 rats, which was absent in intact animals ($p<0.05$). Corneal reflex was absent in most animals upon touching their corneas with a tassel ($p<0.05$). Hindlimbs were extended in 8 out of 9 rats. The average intensity of pain syndrome upon tail compression was 1.14±0.06 points, representing a 2.5-fold decrease compared to the control ($p<0.05$).

In all other groups of kindled rats, whether receiving VT alone or in combination with antiepileptic drugs, the expression of the investigated posture behavior syndrome remained unchanged. Notably, only in the group of rats receiving Naloxone, exophthalmos was observed in 1 out of 8 rats, a normal corneal reflex and tail tone were evident in 5 out of 8 rats, and the average intensity of the pain reaction exceeded that of kindled rats by 2.3 times ($p<0.05$) in all instances.

Upon delving into the nature of posture reactions among kindled rats, significant alterations in the components of this behavior were detected solely within the group of kindled rats receiving both VPA and VT (Table 2). Within this context, exophthalmos was present in 1 out of 6 rats, a normal tail tone was registered in 5 out of 6 rats, 4 out of 6 rats were capable of maintaining the ‘bridge’ posture, none of the rats could cling to the vertical pole, and the average intensity of the pain reaction was 30.5% lower compared to kindled rats ($p<0.05$) in all cases.

Comparison with the indices of posture behavior in intact and kindled rats receiving both VPA and VT demonstrated that similar results were obtained when administering Atropine to kindled rats.

Table 1

Impact of Vortioxetine and antiepileptic drugs combined administration on posture behavior in rats on the 18th day of Picrotoxin-induced chronic seizure syndrome

Animal Groups	Investigated Parameters										
	Position of forelimbs	Flipping reflex	Ocular symptoms	Corneal reflex	Forced posture	Tail tone	Forelimb clasp	"Bridge"	Holding on "vertical grid"	Pain reflex	Explosiveness
1	2	3	4	5	6	7	8	9	10	11	12
1. Control, n=11	Spread forelimbs, n=10	Normal, n=11	Exophthalmos, n=0	Normal, n=10	Hindlimbs extended, n=10	Normal, n=11	n=9	n=8	n=0	2.82±0.12	n=0
2. Kindling, n=9	Spread forelimbs, n=5	Impaired, n=8	Exophthalmos, n=8#	Absent, n=8#	Hindlimbs extended, n=8	High, n=7#	n=7	n=9	n=1	1.14±0.06*	n=3
3. Kindling + VPA, n=6	Spread forelimbs, n=5	Impaired, n=4	Exophthalmos, n=3	Absent, n=4#	Hindlimbs extended, n=5	High, n=4#	n=3	n=5	n=0	1.37±0.12*	n=2
4. Kindling + DPH, n=6	Spread forelimbs, n=	Impaired, n=5	Exophthalmos, n=5#	Absent, n=5#	Hindlimbs extended, n=4	High, n=5#	n=3	n=4	n=0	1.22±0.12*	n=3
5. Kindling + PB, n=6	Spread forelimbs, n=4#	Impaired, n=5	Exophthalmos, n=5#	Absent, n=5#	Hindlimbs extended, n=5	High, n=5#	n=5	n=2	n=1	1.19±0.11*	n=4
6. Kindling + VT, n=6	Spread forelimbs, n=3	Impaired, n=5	Exophthalmos, n=5#	Absent, n=4#	Hindlimbs extended, n=5	High, n=5#	n=5	n=4	n=2	1.36±0.14*	n=3
7. Kindling + VPA+ VT, n=6	Spread forelimbs, n=3	Impaired, n=4	Exophthalmos, n=3	Absent, n=4#	Hindlimbs extended, n=6	High, n=4#	n=6	n=5	n=3	1.32±0.08*	n=3
8. Kindling + DPH+ VT, n=6	Spread forelimbs, n=5	Impaired, n=5	Exophthalmos, n=4#	Absent, n=5#	Hindlimbs extended, n=5	High, n=5#	n=4	n=1	n=0	1.19±0.12*	n=4

1	2	3	4	5	6	7	8	9	10	11	12
9. Kindling + PB+ VT, n=6	Spread forelimbs, n=5	Impaired, n=5	Exophthalmos, n=5#	Absent, n=5#	Hindlimbs extended, n=5	High, n=5#	n=4	n=2	n=1	1.46±0.17*	n=4
10. Kindling + KET, n=8	Spread forelimbs, n=7	Impaired, n=7	Exophthalmos, n=6#	Absent, n=7#	Hindlimbs extended, n=6	High, n=7#	n=7	n=4	n=3	1.46±0.17*	n=4
11. Kindling + ATR, n=8	Spread forelimbs, n=8	Impaired, n=6	Exophthalmos, n=6#	Absent, n=6#	Hindlimbs extended, n=7	High, n=6#	n=6	n=3	n=3	1.46±0.17*	n=4
12. Kindling + NAL, n=8	Spread forelimbs, n=8	Impaired, n=6	Exophthalmos, n=1@	Normal, n=5@	Hindlimbs extended, n=7	Normal, n=5@	n=5	n=3	n=3	2.61±0.21@@	n=0

Notes (in tables 1-6):

* - $p<0.05$ – probable discrepancies of investigated parameters compared to data in the control group of animals (ANOVA + Newman-Keuls criterion);

- $p<0.05$ – probable discrepancies of investigated parameters compared to data in the control group of animals (Kruskal-Wallis criterion);

@ - $p<0.05$ – probable discrepancies of investigated parameters compared to data in kindled rats (Kruskal-Wallis criterion);

@@ - $p<0.05$ – probable discrepancies of investigated parameters compared to data in kindled rats (ANOVA + Newman-Keuls criterion).

Table 2

Impact of Vortioxetine and antiepileptic drugs combined administration on posture behavior in kindled rats

Animal Groups	Investigated Parameters										
	Position of forelimbs	Flipping reflex	Ocular symptoms	Corneal reflex	Forced posture	Tail tone	Forelimb clasp	"Bridge"	Holding on "vertical grid"	Pain reflex	Explosive ness
1	2	3	4	5	6	7	8	9	10	11	12
1. Control, n=11	Spread forelimbs, n=6	Normal, n=8	Exophthal mos, n=0	Normal, n=8	Hindlimbs extended, n=9	Normal, n=9	n=7	n=7	n=0	2.58±0.12	n=0
2. Kindling, n=9	Spread forelimbs, n=9	Normal, n=6	Exophthal mos, n=5#	Normal, n=9	Hindlimbs extended, n=9	Low, n=7#	n=7	n=2#	n=4#	3.84±0.21*	n=4#
3. Kindling + VPA, n=6	Spread forelimbs, n=4	Normal, n=4	Exophthal mos, n=3	Normal, n=5	Hindlimbs extended, n=5	Low, n=3	n=4	n=3	n=3#	3.21±0.26	n=2
4. Kindling + DPH, n=6	Spread forelimbs, n=6	Normal, n=4	Exophthal mos, n=4#	Normal, n=3	Hindlimbs extended, n=5	Low, n=5#	n=5	n=2#	n=3#	3.76±0.22*	n=3#
5. Kindling + PB, n=6	Spread forelimbs, n=6	Normal, n=5	Exophthal mos, n=4#	Normal, n=4	Hindlimbs extended, n=5	Low, n=5#	n=5	n=3	n=3#	3.52±0.23*	n=4#
6. Kindling + VT, n=6	Spread forelimbs, n=4	Normal, n=5	Exophthal mos, n=3	Normal, n=5	Hindlimbs extended, n=5	Low, n=3	n=4	n=3	n=2	3.16±0.23	n=3
7. Kindling + VPA+VT, n=6	Spread forelimbs, n=3	Normal, n=3	Exophthal mos, n=1@	Normal, n=5	Hindlimbs extended, n=6	Normal, n=5@	n=2	n=4@	n=0@	2.67±0.17 @@	n=1
8. Kindling + DPH+VT, n=6	Spread forelimbs, n=5	Normal, n=5	Exophthal mos, n=4#	Normal, n=3	Hindlimbs extended, n=4	Low, n=5#	n=6	n=3	n=4#	3.66±0.23*	n=4#

1	2	3	4	5	6	7	8	9	10	11	12
9. Kindling + PB+VT, n=6	Spread forelimbs, n=5	Normal, n=6	Exophthalmos, n=4#	Normal, n=5	Hindlimbs extended, n=4	Low, n=6#	n=5	n=3	n=3#	3.63±0.21*	n=5#
10. Kindling + KET, n=8	Spread forelimbs, n=6	Normal, n=7	Exophthalmos, n=6#	Normal, n=5	Hindlimbs extended, n=7	Low, n=6#	n=7	n=3	n=4#	3.57±0.24*	n=4#
11. Kindling + ATR, n=8	Spread forelimbs, n=2	Normal, n=2	Exophthalmos, n=1@	Normal, n=5	Hindlimbs extended, n=6	Normal, n=6@	n=2	n=5@	n=0@	2.51±0.22@@	n=0@
12. Kindling + NAL, n=8	Spread forelimbs, n=7	Normal, n=6	Exophthalmos, n=5#	Normal, n=4	Hindlimbs extended, n=7	Low, n=7#	n=6	n=3#	n=3#	3.49±0.23*	n=5#

Table 3

Impact of Vortioxetine and antiepileptic drugs combined administration on posture behavior in rats in the postkindling stage

Animal Groups	Investigated Parametres										
	Position of forelimbs	Flipping reflex	Ocular symptoms	Corneal reflex	Forced posture	Tail tone	Forelimb clasp	"Bridge"	Holding on "vertical grid"	Pain reflex	Explosive ness
1	2	3	4	5	6	7	8	9	10	11	12
1. Control, n=11	Spread forelimbs, n=9	Normal, n=10	Exophthal mos, n=0	Normal, n=10	Hindlimbs extended, n=9	Normal, n=9	n=10	n=6	n=0	2.88±0.16	n=0
2. Post-Kindling, n=9	Spread forelimbs, n=6	Impaired, n=7#	Exophthal mos, n=6#	Absent, n=7#	Hindlimbs extended, n=7	High, n=6#	n=6	n=8	n=2	1.26±0.09*	n=4#
3. Post-Kindling + VPA, n=6	Spread forelimbs, n=4	Impaired, n=5#	Exophthal mos, n=4#	Absent, n=5#	Hindlimbs extended, n=5	High, n=4#	n=4	n=5	n=2	1.41±0.11*	n=3#
4. Post-Kindling + DPH, n=6	Spread forelimbs, n=4	Impaired, n=4#	Exophthal mos, n=3#	Absent, n=4#	Hindlimbs extended, n=4	High, n=5#	n=5	n=4	n=3	1.37±0.13*	n=4#
5. Post-Kindling + PB, n=6	Spread forelimbs, n=5	Impaired, n=5#	Exophthal mos, n=3#	Absent, n=4#	Hindlimbs extended, n=5	High, n=4#	n=4	n=5	n=2	1.19±0.12*	n=4#
6. Post-Kindling + VT, n=6	Spread forelimbs, n=6	Normal, n=5@	Exophthal mos, n=1@	Normal, n=4@	Hindlimbs extended, n=4	Normal, n=4@	n=4	n=2	n=1	2.33±0.19 @@	n=1@
7. Post-Kindling + VPA+VT, n=6	Spread forelimbs, n=5	Normal, n=5@	Exophthal mos, n=0@	Normal, n=5@	Hindlimbs extended, n=5	Normal, n=5@	n=5	n=3	n=0	2.54±0.21 @@	n=0@
8. Post-Kindling + DPH+VT, n=6	Spread forelimbs, n=3	Impaired, n=4#	Exophthal mos, n=4#	Absent, n=3#	Hindlimbs extended, n=4	High, n=4#	n=4	n=5	n=3	1.39±0.14*	n=4#

1	2	3	4	5	6	7	8	9	10	11	12
9. Post-Kindling + PB+VT, n=6	Spread forelimbs, n=4	Impaired, n=3#	Exophthalmos, n=5#	Absent, n=4#	Hindlimbs extended, n=4	High, n=4#	n=5	n=4	n=3	1.17±0.11*	n=5#
10. Post-Kindling + KET, n=8	Spread forelimbs, n=5	Impaired, n=4#	Exophthalmos, n=5#	Absent, n=6#	Hindlimbs extended, n=5	High, n=5#	n=5	n=5	n=4	1.39±0.13*	n=6#
11. Post-Kindling + ATR, n=8	Spread forelimbs, n=6	Impaired, n=3#	Exophthalmos, n=6#	Absent, n=5#	Hindlimbs extended, n=4	High, n=5#	n=6	n=4	n=4	1.43±0.16*	n=5#
12. Post-Kindling + NAL, n=8	Spread forelimbs, n=7	Normal, n=7@	Exophthalmos, n=0@	Normal, n=8@	Hindlimbs extended, n=7	Normal, n=7@	n=7	n=4	n=0	2.71±0.24 @@	n=0@

Upon investigating changes in post-kindled rats' posture behavior, it was ascertained that behavioral manifestations pertaining to the intensity of the flipping reflex, absence of exophthalmos, presence of corneal reflex, forced posture, tail tone, pain reaction, and explosive behavior mirrored those of intact rats (Group 1), post-kindling stage rats receiving VT (Group 6), post-kindling stage rats receiving both VPA and VT (Group 7), and post-kindling stage rats receiving NAL (Group 12; in all cases $p<0.05$; Table 3).

2. Pharmacological Correction of Emotional Behavior

Throughout the entire study – on the 18th and 24th days of PTX administration, as well as in the post-kindling stage – intact rats exhibited aversive responses to researchers' attempts to handle them. They exhibited intense vocalizations and attempted to bite approaching hands (Fig. 1). Similar emotional behavior was characteristic for kindled rats.

On the 18th day of the study, the average intensity of emotional behavior in kindled rats was significantly reduced after combined administration of VPA and VT (Group 7) and NAL (Group 12) compared to the respective indicator in kindled rats ($p<0.05$).

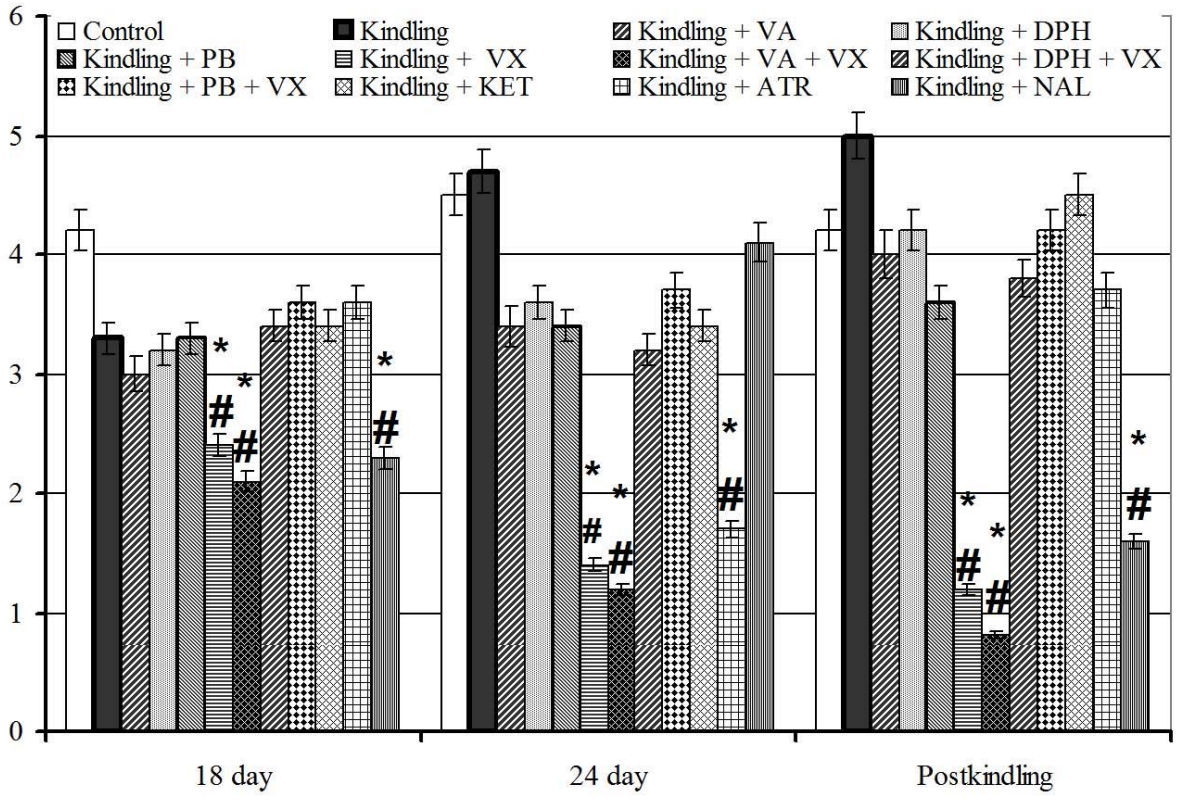

Fig. 1. Impact of combined administration of Vortioxetine and antiepileptic drugs on the expression of emotional behavior in kindled rats.

Notes: * - $p<0.05$ – probable discrepancies of investigated parameters compared to data in the control group of animals;

- $p<0.05$ – probable discrepancies of investigated parameters compared to data in kindled rats (all calculations utilized ANOVA + Newman-Keuls criterion).

On the 24th day of the study, the assessed parameter in kindled rats was 2.8-3.9 times higher compared to those in kindled rats receiving VT (Group 6), combined administration of VPA and VT (Group 7), and ATR (Group 11; in all cases $p<0.05$).

During the post-kindling stage, the average intensity of emotional behavior across all investigated experimental groups was notably lower in rats receiving VT (Group 6; by a factor of 4.2), VT combined with VPA (Group 7; by a factor of 6.25), and ATR (Group 11; by a factor of 3.1) compared to the indicator in other rats ($p<0.05$).

3. Pharmacological Correction of Swimming Behavior

Upon placement in a water basin, intact rats of the Control group displayed a single passive-adaptive swimming behavior act each (swimming along the wall or 'paddling' in the center of the basin or 'paddling' while holding onto the basin wall). Conversely, the remaining rats exhibited more than 3 passive-adaptive swimming behavior acts. Consequently, the average number of passive-adaptive acts in the Control group rats was recorded at 2.19±0.27, and the variability index was 44% (Table 4). After swimming, a rope was introduced into the basin for the Control group rats; three animals ascended after visual contact, while the remainder ascended after snout contact with the rope.

Table 4

Impact of Vortioxetine and antiepileptic drugs combined administration on swimming behavior in rats on the 18th day of picrotoxin-induced chronic seizure syndrome

Animal Groups	Investigated Parametres			
	Number of passive-adaptive acts, M±m	Variability index, %	Maximum variability index, %	Ability to switch to active-adaptive swimming behavior
1. Control, n=9	2.19±0.27	44	0	0.67±0.06
2. Kindling, n=11	3.27±0.34	72#	0	1.41±0.11*
3. Kindling + VPA, n=6	2.54±0.22	50	0	1.38±0.13*
4. Kindling + DPH, n=6	2.87±0.26	67	0	1.12±0.12*
5. Kindling + PB, n=6	3.08±0.27	67	0	1.54±0.16*
6. Kindling + VT, n=6	2.46±0.23	50	0	0.73±0.06@@
7. Kindling + VPA+VT, n=6	2.37±0.23	33@	0	0.52±0.05@@
8. Kindling + DPH+VT, n=6	2.74±0.27	67	0	1.46±0.16*
9. Kindling + PB+VT, n=6	2.69±0.28	67	0	1.29±0.13*
10. Kindling + KET, n=8	2.51±0.26	75#	0	1.36±0.17*
11. Kindling + ATR, n=8	2.72±0.27	63	0	1.24±0.13*
12. Kindling + NAL, n=8	2.29±0.24	38@	0	0.56±0.06@@

After the 18th administration of PTX, two rats displayed two passive-adaptive swimming behavior acts, while the remaining rats exhibited more than three. The average number of passive-adaptive acts under these conditions was comparable to the corresponding indicator in intact rats ($p>0.05$), while the variability index was higher than that in the control ($p<0.05$). Subsequent to swimming, five rats exited the basin after contacting the rope with their snouts and front paws, while six rats exited after contacting the rope with their snouts, front, and hind paws. The degree of contact with the rope required for exiting the basin in kindled rats after the 18th administration of PTX was 2.1 times greater than the corresponding control indicator ($p<0.05$).

The normalization of the variability index and the degree of contact with the rope in rats after the 18th administration of PTX were analogous to the corresponding control measurements in the case of combined administration of VPA and VT, as well as Naloxone administration ($p<0.05$).

The collected data suggest the normalization of the investigated indicators in the number of passive-adaptive swimming behavior acts, variability index, and the ability to transition to active-adaptive swimming behavior in kindled rats after the administration of VT (Group 6), combined administration of VPA and VT (Group 7), and Atropine administration (Group 11; in all cases $p<0.05$; Table 5).

Table 5

Impact of Vortioxetine and antiepileptic drugs combined administration on swimming behavior in kindled rats

Animal Groups	Investigated Parametres			
	Number of passive-adaptive acts, M±m	Variability index, %	Maximum variability index, %	Ability to switch to active-adaptive swimming behavior
1. Control, n=9	2.37±0.26	44	0	0.81±0.07
2. Kindling, n=11	4.71±0.38*	91#	36#	2.87±0.26*
3. Kindling + VPA, n=6	3.67±0.29*	67	33#	2.19±0.19*
4. Kindling + DPH, n=6	3.91±0.32*	83#	50#	2.48±0.24*
5. Kindling + PB, n=6	4.11±0.33*	83#	50#	2.96±0.28*
6. Kindling + VT, n=6	2.56±0.24@@	33@	17	1.07±0.09@@
7. Kindling + VPA+VT, n=6	2.29±0.27@@	17@	0@	0.89±0.09@@
8. Kindling + DPH+VT, n=6	3.74±0.33*	67	50#	2.59±0.26*
9. Kindling + PB+VT, n=6	4.08±0.37*	83#	33#	2.93±0.29*
10. Kindling + KET, n=8	3.59±0.36*	50	38#	2.66±0.24*
11. Kindling + ATR, n=8	2.38±0.26@@	38@	0@	0.96±0.09@@
12. Kindling + NAL, n=8	3.82±0.37*	63	38#	2.72±0.27*

In rats at the post-kindling stage, the number of passive-adaptive swimming behavior acts, variability index, maximum variability index, and the degree of contact with the rope required for

exiting the basin were akin to the corresponding indicators in intact rats solely in the groups after combined administration of VPA and VT and Naloxone administration ($p<0.05$; Table 6).

Table 6

Impact of Vortioxetine and antiepileptic drugs combined administration of on swimming behavior in postkindled rats

Animal Groups	Investigated Parametres			
	Number of passive-adaptive acts, M±m	Variability index, %	Maximum variability index, %	Ability to switch to active-adaptive swimming behavior
1. Control, n=9	2.27±0.24	44	0	0.86±0.09
2. Postkindling, n=11	3.68±0.29*	64	45#	1.94±0.17*
3. Postkindling + VPA, n=6	2.81±0.26	67	50#	1.48±0.13
4. Postkindling + DPH, n=6	3.41±0.28*	67	50#	1.61±0.14*
5. Postkindling + PB, n=6	3.54±0.29*	67	50#	1.57±0.14*
6. Postkindling + VT, n=6	2.61±0.23	50	33	1.26±0.11@@
7. Postkindling+VPA+VT, n=6	2.19±0.26@@	33@	17@	0.77±0.07@@
8. Postkindling+DPH+VT, n=6	3.36±0.32*	67	50#	1.39±0.16
9. Postkindling + PB + VT, n=6	3.23±0.28*	50	33	1.52±0.16
10. Postkindling + KET, n=8	2.97±0.27*	63	38	1.37±0.14
11. Postkindling + ATR, n=8	3.16±0.29*	50	38	1.47±0.16
12. Postkindling + NAL, n=8	2.34±0.21@@	25@	13@	0.84±0.08@@

Discussion

In conclusion, the acquired data offer insight into the presence of disturbances in certain forms of behavior, categorized as non-seizure behavior, in rats experiencing post-kindling-induced chronic epileptic syndrome. The chosen behavioral dimensions – namely, late tonic, emotional, and swimming behaviors – closely align with the designated research framework, as these behavioral irregularities are commonly observed during interictal periods in cases of kindling-induced chronic epileptogenesis [7-9, 36].

Consequently, during the stages of established and formed kindling, as well as in the heightened seizure susceptibility phase post-kindling, rats demonstrate dysfunctions in late-tonic behavior, predominantly influenced by opioid mechanisms. Emotional behavior becomes heightened, and disruptions are apparent in swimming behavior. These collective disturbances point to intrastriatal dysfunction, compromised relay functioning of the striatum, and a reduced capacity of the brain to transition to active-adaptive behavioral acts. These findings are congruent with analogous behavioral aberrations identified in other forms of chronic epileptogenesis [1-3, 7, 9], which further contribute to the comprehension of mesencephalic dysfunctions [4].

The amassed factual evidence highlights the successful pharmacological correction of the examined behavioral dimensions through the concurrent administration of Valproic acid and Vortioxetine. Nonetheless, the efficacy in mitigating non-seizure behavioral disturbances is less pronounced with the sole application of Vortioxetine. The least degree of effectiveness is observed in the correction of fewer behavioral types following Valproic acid administration.

The data underscores the restoration of non-seizure behavior anomalies during the developed kindling and post-kindling stages when Naloxone is introduced to rats. A parallel effect is evident during the formed kindling stage with the administration of Atropine, which suggests an opiate neurotransmitter mechanism influencing non-seizure behavioral disruptions in the developed kindling and post-kindling stages, and a cholinergic mechanism in fully kindled rats. The discussion surrounding these outcomes highlights the prevalence of opiate neurotransmission following the 18th kindling induction and during the post-kindling phase, along with the dominance of cholinergic neurotransmission in fully kindled rats post the 24th kindling induction. Throughout kindling formation, functional enhancement of cholinergic and dopaminergic neurotransmission in the striatum, coupled with the suppression of GABA mechanisms, is noted. This serves as an instance of the functional interplay between epileptogenic and antiepileptogenic systems [4, 31].

In evaluating the obtained data, certain focal points merit consideration. The current findings align with our prior affirmative outcomes from the joint application of Valproic acid and Vortioxetine in terms of ameliorating cognitive impairments during the stages of developed and formed post-kindling-induced chronic epileptic syndrome, as well as the post-kindling phase [4]. Consequently, there is substantial foundation to regard these findings as experimental rationale for the clinical examination of the combined effects of VT with VA in epilepsy patients displaying behavioral anomalies.

Within this context, it is worth noting that the multi-faceted antidepressant VT, employed in this study, exhibits a spectrum of promising effects, encompassing anxiolytic and procognitive impacts, reduction in obsessiveness and compulsiveness, in addition to addressing suicidal tendencies [6]. It's pertinent to highlight that VT, in its antidepressant action, displays greater selectivity towards the 5-HT1A receptor – a central subtype of serotonin receptors implicated in the pathogenesis of depression [29, 32]. Building upon this component of the findings, it can be inferred that the heightened non-seizure behavior witnessed in the progression of post-kindling-induced chronic epileptic syndrome is indicative of an underlying depressive nature within the animals' behavioral milieu. This inference is substantiated by other researchers under experimental conditions [5, 9] and is mirrored in clinical observations within neurological clinics [16, 17, 22].

Finally, contemplating the substantial comorbidity between epilepsy and the frequent occurrence of depressive behavioral manifestations within the clinical presentation of chronic

epileptic syndrome, a pivotal question emerges: should immediate seizure reactions and manifestations take precedence over comorbid disorders and complications in treatment? These accompanying issues progressively erode the quality of life for patients, impact the clinical expression of the primary disorder, and contribute to heightened seizure frequency. We believe it is pertinent to concur with the prevailing viewpoint advocating for a comprehensive pharmacological approach. This approach should be grounded in pathogenic rationale, emphasizing the simultaneous administration of pharmacological agents with established antiseizure and antidepressant properties. This supposition finds validation in our data, where a clear tendency towards the restoration of disrupted non-seizure behavioral forms during interictal periods of chronic epileptic syndrome is evident under the influence of Valproic acid (to a greater extent) and Fluoxetine (to a lesser extent).

In summation, we underscore that the acquired data unveil a delicate equilibrium within systemic-antisynergistic interactions, the perturbation of which during the progression of post-kindling-induced chronic epileptic syndrome fosters the emergence of non-seizure behavior forms during interictal intervals. The chosen behavioral dimensions and their disruptions offer insights into the interplay between opiate and cholinergic neurotransmitter mechanisms in shaping the kindling syndrome. The experimental outcomes and their analytical interpretation advocate for the clinical exploration of combined Valproic acid and Vortioxetine administration as an integral facet of comprehensive therapy for epilepsy patients manifesting depressive behavioral components.

Conclusions

1. Rats undergoing the course of Picrotoxin-induced chronic epileptic syndrome exhibit disruptions across late-tonic, emotional, and swimming behavior categories, emblematic of non-seizure behavior manifestations.

2. During the phases of developed and formed kindling, alongside the post-kindling stage, rats manifest perturbations in late-tonic behavior, prominently influenced by opioid mechanisms. This period also witnesses heightened emotional behavior and disturbances in swimming proficiency. Collectively, these observations signify intrastriatal dysfunction, compromised relay functionality within the striatum, and a constrained capacity for the brain to transition to active-adaptive behavioral responses.

3. The combined administration of Valproic acid and Vortioxetine results in efficacious correction of the evaluated behavioral dimensions. Comparatively, Vortioxetine alone exhibits lesser effectiveness, and Valproic acid administration yields the least degree of impact on a more limited array of examined behavioral types.

4. Evidence substantiates an opiate-mediated mechanism contributing to the emergence of non-seizure behavioral disruptions during the developed kindling and post-kindling stages, while

a cholinergic mechanism plays a role during the fully formed kindling stage. These findings illuminate the equilibrium within systemic-antisynergistic interactions, the disturbance of which during the course of Picrotoxin-induced chronic epileptic syndrome facilitates the development of non-seizure behavior manifestations during interictal intervals.

5. The amassed data and ensuing analysis furnish experimental rationale for the feasibility of clinical trials examining the conjoined use of Vortioxetine and Valproic acid within comprehensive therapy for epilepsy patients grappling with concurrent depressive behavioral components.

References

1. Vastyanov R.S. Pathophysiological mechanisms of epileptic activity in chronic epilepsy (experimental study). Abstr. Doctor of Science degree. Speciality. 14.03.04 “Pathological physiology”. Odesa. 2013: 35 [In Ukrainian].

2. Vastyanov R.S., Oliynyk A.A., Kashchenko O.A. Changes in the behavior of rats during swimming with pilocarpine convulsions. Odesa Medical Journal. 2002; 6: 11-15 [In Ukrainian].

3. Vastyanov R.S., Kopyova N.V. Peculiarities of the emotional behavior of rats in the dynamics of the development of pilocarpine-induced spontaneous seizures. Ukrainian medical almanac. 2009; 12(1): 28–33 [In Ukrainian].

4. Ostapenko I.O. The effect of brintellix on the expressiveness of memory and learning processes under conditions of chronic convulsive syndrome. Herald of marine medicine. 2022; 2(95): 38-51 [In Ukrainian].

5. Prishchepa E.A. Changes in motor, exploratory activity and emotional behavior of animals under conditions of long-term chemical kindling. Herald of psychiatry and psychopharmacotherapy. 2012; 2: 30-38 [In Russian].

6. Shagiakhmetov F.Sh., Anokhin P.K., Shamakina I.Yu. Vortioxetine: Mechanisms of Multimodality and Clinical Efficacy. Social and Clinical Psychiatry. 2016; 26(4): 84-96 [In Russian].

7. Shandra A.A., Godlevsky L.S., Mazarati A.M. Kindling as a model for the formation of behavioral disorders. Uspekhi Fiziol. Sciences. 1990; 21 (4): 50-68 [In Russian].

8. Shandra A.A., Godlevsky L.S., Mazarati A.M. Formation of motor programs during pharmacological kindling. I.M. Sechenov Fiziol. J. USSR. 1990; 76(10): 1384-1392 [In Russian].

9. Shandra A.A., Godlevsky L.S., Brusentsov A.I. Kindling and epileptic activity. Odessa : Astroprint. 1999: 191 [In Russian].

10. Akanuma N., Hara E., Adachi N., Hara K., Koutroumanidis M. Psychiatric comorbidity in adult patients with idiopathic generalized epilepsy. Epilepsy Behav. 2008; 13(1): 248–251.

11. Anhoury S., Brown R.J., Krishnamoorthy E.S., Trimble M.R. Psychiatric outcome after temporal lobectomy: a predictive study. Epilepsia. 2000; 41: 1608-1615.

12. Aycicek A., Iscan A. The effects of carbamazepine, valproic acid and phenobarbital on the oxidative and antioxidative balance in epileptic children. Eur. Neurol. 2007; 57(2): 65–69.

13. Barry J.J., Lembke A., Huynh N. Affective disorders in epilepsy. Psychiatric Issues in Epilepsy: A Practical Guide to Diagnosis and Treatment. Ed. by Ettinger A., Kanner A. Philadelphia: Lippincott/Williams and Wilkins. 2001: 45-71.

14. Cardamone L., Salzberg M.R., O'Brien T.J., Jones N.C. Antidepressant therapy in epilepsy: can treating the comorbidities affect the underlying disorder? Br J Pharmacol. 2013; 168(7): 1531–1554.

15. Claycomb R.J., Hewett S.J., Hewett J.A. Prophylactic, prandial rofecoxib treatment lacks efficacy against acute PTZ-induced seizure generation and kindling acquisition. Epilepsia. 2011; 52(2): 273–283.

16. Dudra-Jastrzebska M., Andres-Mach M.M., Luszczki J.J., Czuczwar S.J. Mood disorders in patients with epilepsy. Pharmacol. Rep. 2007; 59: 369–378.

17. Ettinger A.B., Weisbrot D.M., Nolan E.E., Gadow K.D., Vitale S.A., Andriola M.R., Lenn N.J., Novak G.P., Hermann B.P. Symptoms of depression and anxiety in pediatric epilepsy patients. Epilepsia. 1998; 39: 595-599.

18. Frucht M.M., Quigg M., Schwaner C., Fountain N.B. Distribution of seizure precipitants among epilepsy syndromes. Epilepsia. 2000; 41: 1534-1539.

19. Hamid H., Kanner A.M. Should antidepressant drugs of the selective serotonin reuptake inhibitor family be tested as antiepileptic drugs? Epilepsy Behav. 2013; 26(3): 261-265.

20. Jones J.E., Hermann B.P., Barry J.J., Gilliam F.G., Kanner A.M., Meador K.J. Rates and risk factors for suicide, suicidal ideation, and suicide attempts in chronic epilepsy. Epilepsy Behav. 2003; 4(3): 31–38.

21. Kanner A.M. The treatment of depressive disorders in epilepsy: what all neurologists should know. Epilepsia. 2013; 54: 3–12.

22. La France W.C. Jr, Kanner A.M., Hermann B. Psychiatric comorbidities in epilepsy. Neurobiology. 2008; 83: 347–383.

23. Maguire M.J., Marson A.G., Nevitt S.J. Antidepressants for people with epilepsy and depression. Cochrane Database Syst Rev. 2021; 4(4): CD010682. doi: 10.1002/14651858.CD010682.pub3.

24. Myslobodsky M., Kofman O., Mintz M. Convulsant specific architecture of postictal behavioral syndrome in the rat. Epilepsia. 1981; 27(4): 559–568.

25. Nestler E.J., Barrot M., Di Leone R.J., Eisch A.J., Gold S.J, Monteggia L.M. Neurobiology of depression. Neuron. 2002; 34(1): 13–25.

26. Ostapenko I.O. The perspectives of pharmacological correction of depressive disorders and cognitive deficit as post-traumatic epilepsy leading syndromes. Journal of Education, Health and Sport. 2022; 12(2): 360-369.

27. Post R.M., Squillace K.M., Pert A., Sass W. The effect of amygdala kindling on spontaneous and cocaine-induced motor activity and lidocaine seizures. Psychopharmacology (Berl). 1981; 72(2): 189-196.

28. Pressler R.M., Lagae L. Why we urgently need improved seizure and epilepsy therapies for children and neonates. Neuropharmacology. 2020; 170: 107854. doi: 10.1016/j.neuropharm.2019.107854.

29. Riga M.S., Sánchez C., Celada P., Artigas F. Involvement of 5-HT3 receptors in the action of vortioxetine in rat brain: Focus on glutamatergic and GABAergic neurotransmission. Neuropharmacology. 2016; 108: 73–81.

30. Shandra A.A., Mazarati A.M., Godlevsky L.S., Vastyanov R.S. Chemical Kindling: Implications for Antiepileptic Drugs-Sensitive and Resistant Epilepsy Model. Epilepsia. 1996; 37(3): 269-274.

31. Shandra A.A., Godlevsky L.S., Vastyanov R.S. Epileptic and antiepileptic systems interrelation as the systemic indicator of the complexity of epileptic activity manifestation. Pan-Brain Abnormal Neural Network in Epilepsy. Ed. by Feng Ru Tang. Singapore : Research Signpost, 2009. 99-120.

32. Stenkrona P., Halldin C., Lundberg J. 5-HTT and 5-HT1A receptor occupancy of the novel substance vortioxetine (Lu AA21004).A PET study in control subjects. Eur. Neuropsychopharmacol. 2013; 23: 1190–1198.

33. Thijs R.D., Surges R., O'Brien T.J., Sander J.W. Epilepsy in adults. Lancet. 2019; 393(10172): 689-701.

34. Thapar A., Kerr M., Harold G. Stress, anxiety, depression, and epilepsy: Investigating the relationship between psychological factors and seizures. Epilepsy Behav. 2009; 14: 134–140.

35. Vastyanov R.S., Stoyanov O.M., Platonova O.M., Yermuraki P.P., Ostapenko I.O., Tatarko S.V., Bibikova V.M. Pathogenetic mechanisms of convulsive depressive syndrome in conditions of kindling model of epileptogenesis. World of medicine and biology. 2021; 1(75): 181-186.

36. Vastyanov R.S., Kirchev V.V., Muratova T.M., Kashchenko O.A., Vastyanova O.V., Tatarko S.V., Zayats L.M. Comparative analysis of motor and emotional behavioral disorders in conditions of experimental chronic ischemic and chronic convulsive syndromes. World of medicine and biology. 2021; 2(76): 183-188.

37. Vrijmoed-de Vries M.C., Cools A.R. Differential effects of striatal injections of dopaminergic, cholinergic and GABAergic drugs upon swimming behavior of rats. Brain Res. 1986; 364(1): 77-90.

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- conceptualization, methodology, formal analysis, data curation, writing—original draft preparation, writing—review and editing & supervision.

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Informed Consent Statement

The data of experimental studies are given. Written informed consent from the patients was not necessary to publish this paper.

Data Availability Statement

The data presented in this study are available on request from the author.

Conflicts of Interest

There is no conflict of interest.