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COGNITIVE FUNCTION RESTORATION IN RATS WITH CHRONIC BRAIN ISCHEMIA USING SEMAX AND HOPANTENIC ACID COMPREHENSIVE ADMINISTRATION

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Abstract

The relevance and importance of treating patients with ischemic brain lesions are indisputable. Chronic ischemic processes in the brain occur much more frequently than acute cerebral circulation disorders, leading to prolonged disability and being the main contributors to a significant number of strokes. Cognitive dysfunctions prevail in the dynamics of the post-ischemic period and are often the primary determinants of the severity of residual organic and subsequent functional impairments of the brain. The aim of the study is to investigate the effectiveness of separate and combined administration of Semax and hopantenic acid in the pharmacological correction of cognitive disorders in rats with experimental chronic brain ischemia. It has been established that rats with chronic brain ischemia exhibit cognitive impairments from the 1st day, manifested in worsened learning processes, short-term and long-term memory functioning in the context of conditioned active avoidance and conditioned feeding reflexes. It has been shown that separate and combined application of Semax and hopantenic acid contributes to the restoration of the learning process and improvement of

mnesic functions in rats with chronic brain ischemia. The most pronounced nootropic effect in rats with chronic brain ischemia was registered under the conditions of combined administration of Semax and hopantenic acid, starting from the 3rd day of the study. Subsequently, in terms of anti-ischemic efficacy, the effect of Semax follows, which was realized starting from the 3rd day of the study. The least pronounced neuroprotective effect belongs to hopantenic acid, starting from the 7th day of the study. The author considers the obtained data as experimental groundwork for the feasibility of clinically testing the nootropic effects of the combined administration of Semax and hopantenic acid in cases of chronic brain ischemia. The comprehensive pharmacological correction of cognitive disorders in chronic brain ischemia is pathogenetically justified and aimed at activating sanogenetic mechanisms.

Key words: chronic brain ischemia; Semax; hopantenic acid; learning; short-term memory; conditioned-reflectory activity

The significance and importance of treating patients with cerebral ischemic brain damage are indisputable. This assertion is supported by available statistical data, indicating a steady annual increase in the number of individuals diagnosed with strokes not only in our country but also across Western European countries and worldwide [1, 7, 18]. It is noteworthy that chronic cerebral ischemic processes occur more frequently than acute ones, leading to prolonged disability and a considerable incidence of cerebral strokes [1, 6].

Although adhering to all the requisites of modern treatment protocols for this patient population typically results in a positive prognosis for survival [1, 18], in our perspective, an essential concern remains regarding the restoration of organs, systems, and specific body functions with regulatory, adaptive, and/or compensatory attributes [1, 19].

From both therapeutic and neurological viewpoints, the challenge lies in the fact that complete restoration of organism functions during the post-ischemic period is not invariably achieved, thereby diminishing the quality of life for patients [6, 17]. In this context, the treatment of chronic forms of cerebral blood supply insufficiency becomes particularly pertinent [1, 6, 14]. From a pathophysiological standpoint, the emphasis rests on the observation that even with the restoration of vital functions, recovery of those responsible for swift and efficient adaptation of the organism to diverse environmental conditions is often partial or inadequate [1, 19]. From a neuropharmacological perspective, the objective is to amplify the efficacy and capacity of secondary neuroprotection to attain as comprehensive restoration of all organism functions as feasible [18].

Foundational studies have elucidated that the pathogenetic mechanisms of ischemic neuronal injury encompass a cascade of intricate chains that are activated simultaneously and sequentially, entailing destructive and necrotic alternative pathologies in physiological, biochemical, and morphological processes [1, 27]. It is worth noting that pronounced motor dysfunctions develop during the course of chronic brain ischemia (CBI), manifested as pyramidal and extrapyramidal neurological disorders [5, 6]. Furthermore, significant disruptions manifest in the regulatory brain processes governed by vestibular, visual, cutaneous, proprioceptive, and other sensory cortical projections, along with central control mechanisms [7, 9, 17, 18, 24].

The progressive emergence of cognitive dysfunctions, impairment of learning processes, short-term and long-term memory deficits, disruptions in visual, spatial, referential, and working memory, and associated emotional state disturbances are closely correlated with the advancement of CBI [1, 9, 16]. Consequently, during the post-ischemic period, mnemonic dysfunctions predominate, frequently determining the severity of residual organic and functional brain impairments [1, 9, 16, 23]. Therefore, we consider their treatment to be a pivotal avenue in secondary neuroprotection, as well as a preventive, pathogenetically justified pharmacological intervention to address potential non-motor complications resulting from CBI.

Our prior experimental trials revealed Semax and hopantenic acid combined administration effectiveness in rectifying motor disorders and neurological deficiencies in CBI-induced rats. Considering this, along with the frequently prevailing clinical scenario of mnemonic dysfunctions stemming from the post-ischemic state, we embarked on evaluating the efficacy of an original treatment regimen to assess the vigor of learning and memory processes in experimentally induced CBI.

The aim of the work is to scrutinize the efficacy of Semax and hopantenic acid separate and combined administration in pharmacologically correcting cognitive impairments in rats subjected to experimentally induced chronic brain ischemia.

Materials and methods

The research was conducted through controlled experimentation involving 110 male Wistar rats weighing 180-250 g, maintained on a standard diet. The rats were granted unrestricted access to food and water and were housed under standard conditions, adhering to a natural 12-hour light-dark cycle, 60% humidity, and a temperature of $22\pm 1^{\circ}\text{C}$. The handling of laboratory animals adhered to universally accepted protocols for conducting laboratory and other studies involving experimental animals of various species.

The CBI model was induced through a procedure involving the dissection of the skin, isolation, and bilateral ligation of the carotid arteries [13]. The animals were categorized into distinct groups as follows: Group 1 – Control (intact rats with only a skin incision without carotid artery ligation, n=7); Group 2 – Experimental (rats with carotid artery ligation and reconstruction of CBI, n=12); Group 3 – CBI rats treated with Semax (SEM; 0.1% solution, administered intranasally, 10 µl, n=12); Group 4 – CBI rats treated with hopantenic acid (HA; RIK-pharm Ltd, administered intraperitoneally at 100 mg/kg, n=12); Group 5 – CBI rats treated with a combination of SEM and HA (n=12).

Two series of experiments were conducted to evaluate mnemonic functions. These experiments involved assessing the rats' capacity to develop and retain a conditioned active avoidance reflex (CAAR) as well as a conditioned food reflex.

The CAAR development and the evaluation of mnemonic functions were performed in a rectangular chamber measuring 50x15 cm with metallic walls rising to a height of 40 cm, along with a metallic floor linked to an electrical current source. The chamber was partitioned into two equal sections, each measuring 25x15 cm, and separated by a manually operable door. Each section was outfitted with 20 W lamps. The conditioned stimulus (CS) was achieved through light activation, while an electric current of 0.5-0.8 mA was employed as the unconditioned stimulus (US), delivered via the metallic floor. The experimental scheme was presented by [28].

The quantity of conditioned and unconditioned stimuli required for successful learning was quantified in the rats. The retention of learned behavior was evaluated after 24 hours (short-term memory) and 7 days (long-term memory) by presenting the conditioned stimulus followed by the unconditioned one (Fig. 1).

The 'preservation' index was computed as an inclusive indicator of mnemonic functions. This index was calculated as the disparity between the number of pairings between the conditioned stimulus and the unconditioned stimulus needed for skill acquisition and the number of pairings requisite for its replication on the subsequent day. This difference was then divided by the number of pairings necessary for acquiring the skill in the first place.

The characteristics of conditioned food reflex (CFR) development were examined in rats utilizing an eight-arm radial maze (RM), following the methodology outlined by [21]. To induce a heightened food motivation in the rats, they were subjected to food deprivation, resulting in a reduction of their body weight to 85% of their initial weight.

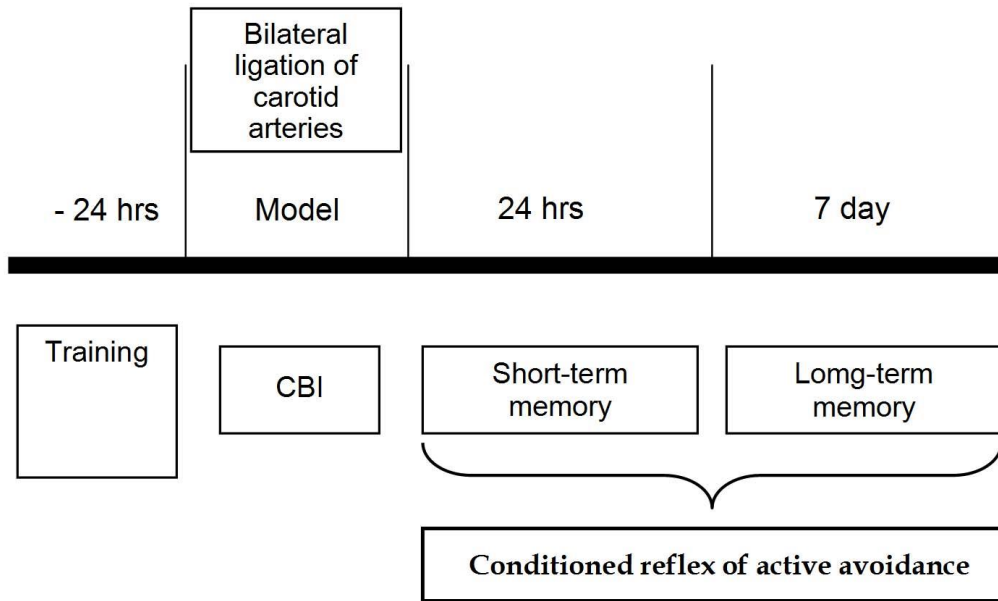


Fig. 1. Approximate scheme of the experimental setup to investigate the impact of the applied treatment regimen on learning and memory processes during the development of the conditioned active avoidance reflex

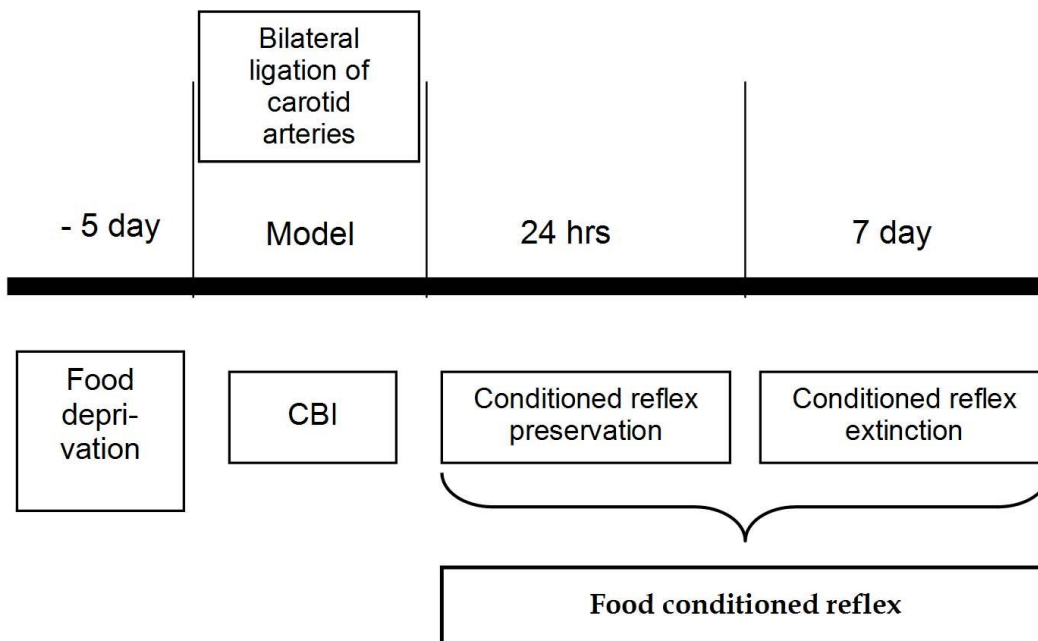


Fig. 2. Approximate scheme of the experimental setup to investigate the impact of the applied treatment regimen on learning and memory processes during the development of the conditioned food reflex

In the series of experiments aimed at gauging the sustenance of previously established conditioned reflexes, trained animals with a well-established habit of locating food in one of the maze arms were employed. The preservation of the conditioned reflex was similarly evaluated one day after the successful development of CFR. Under analogous conditions, separate experiments were conducted to investigate the resistance of the previously established conditioned reflex to extinction. To achieve this, food pellets were deliberately absent in certain maze arms, effectively utilizing negative reinforcement of the conditioned reflex (Fig. 2).

Statistical analysis involved parametric ANOVA and the Newman-Keuls post hoc test for pairwise comparisons, along with the non-parametric Kruskal-Wallis test. The threshold for statistical significance was set at $p < 0.05$.

Results

1. Pharmacological Correction of Cognitive Dysfunctions in Rats with Chronic Brain Ischemia in the Test of Conditioned Active Avoidance Reflex

1.1. Training

At the onset, animals were presented with a conditioned stimulus, followed by an unconditioned stimulus after 5 sec. In response to a series of electric shocks, the animals displayed initial freezing behavior followed by escape attempts. Control group rats required 21-25 sec to reach the goal, with subsequent reduction in escape run time. An avoidance reaction developed, wherein the animal moved to the opposite side upon receiving the CS thus evading the electric stimulus. The criterion for CAAR development was the presence of anticipatory reactions.

From the 1st day after CBI induction, the number of CS and US pairings necessary for CAAR development increased by 43.2% compared to control observations ($p < 0.05$, Table 1). The investigated index in CBI rats treated with SEM and HA was 26.9 ± 2.7 , similar to that in control observations ($p > 0.05$). The value of this index in the other groups exceeded the data from intact rats by 30.7% and 47.5% ($p < 0.05$).

On the 3rd day of the study, in the group of CBI rats treated with HA, the number of CS and US pairings required for CAAR development was 33.4% higher than in intact rats ($p < 0.05$). The investigated indices in CBI rats treated with intranasal SEM and combined SEM and HA administration were comparable to those in the control group and were 31.4% and 35.8% lower, respectively, than the corresponding index in the group of CBI rats without pharmacological correction ($p < 0.05$).

Table 1

Impact of Semax and Hopantenic Acid separate and combined administration on learning processes, short-term and long-term memory in CBI rats

Animal Groups	Number of pairings of conditioned and unconditioned stimuli required for the CAAR development		
	Training	Short-term Memory	Long-term memory
<i>1st day</i>			
1 Group – Control, n=7	22.8±2.4	7.2±0.8	2.8±0.3
2 Group – CBI, n=12	32.7±3.3*	12.3±1.2*	5.7±0.7**
3 Group – CBI + SEM, n=12	29.8±3.2*	10.1±1.1*	4.6±0.4
4 Group - CBI + HA, n=12	33.6±3.4*	11.4±1.2*	5.4±0.5*
5 Group – CBI + SEM + HA, n=12	26.9±2.7	9.3±0.9	4.1±0.4
<i>3rd day</i>			
1 Group – Control, n=7	23.3±2.6	6.9±0.8	2.6±0.3
2 Group – CBI, n=9	38.8±3.7*	16.9±1.7**	8.3±0.7**
3 Group – CBI + SEM, n=10	26.6±2.5#	9.1±0.9#	5.1±0.4##
4 Group - CBI + HA, n=9	31.1±3.1*	10.7±1.1	7.3±0.6**
5 Group – CBI + SEM + HA, n=10	24.9±2.4#	8.4±0.7#	4.0±0.4### @
<i>5th day</i>			
1 Group – Control, n=7	24.3±2.4	6.6±0.7	2.5±0.3
2 Group – CBI, n=8	37.3±3.6*	16.1±1.6**	7.4±0.7**
3 Group – CBI + SEM, n=9	25.9±2.4#	8.6±0.7##	4.7±0.4#
4 Group - CBI + HA, n=9	31.4±2.9*	10.3±0.9	5.8±0.5*
5 Group – CBI + SEM + HA, n=10	25.0±2.4# @	7.3±0.6##	3.5±0.4### @
<i>7th day</i>			
1 Group – Control, n=7	22.9±2.4	6.7±0.8	2.7±0.3
2 Group – CBI, n=8	33.4±3.4*	13.7±1.4*	6.9±0.7*
3 Group – CBI + SEM, n=9	25.2±2.4#	7.9±0.7#	3.6±0.4#
4 Group - CBI + HA, n=9	28.3±2.7	8.9±0.8	4.7±0.4
5 Group – CBI + SEM + HA, n=10	23.4±2.3# @	7.2±0.6##	3.3±0.3### @

Designations. Reduction in the number of rats in groups occurred due to their demise.

Notes. * - $p < 0.05$ and ** - $p < 0.01$ – probable discrepancies in the investigated indicators compared to the data in the control group;

- $p < 0.05$ and ## - $p < 0.01$ – probable discrepancies in the investigated indicators compared to the data in CBI rats without pharmacological correction;

@ - $p < 0.05$ – probable discrepancies in the investigated indicators compared to the data in CBI rats treated with Semax and/or HA (Kruskal-Wallis test applied for all calculations)

On the 5th day of the study, the number of CS and US pairings needed for CAAR development in CBI rats treated with combined SEM and HA was 32.9% lower than in the

group of CBI rats without pharmacological correction and 20.4% lower than in the HA-treated group ($p < 0.05$ in all cases).

Similar trends were observed on the 7th day of the post-ischemic period.

1.2. Short-term memory

The number of CS and US pairings required for CAAR development on the day following CR induction in CBI rats was 70.8% higher compared to control measurements ($p < 0.05$). During this interval, only the group of CBI rats receiving combined SEM and HA administration achieved an investigated index not significantly different from the control group ($p > 0.05$).

The number of CS and US pairings required for CAAR development was significantly reduced by 46.2% and 50.3% in the groups of CBI rats that received intranasal SEM and combined SEM and HA administration, respectively, in comparison to CBI rats without pharmacological correction ($p < 0.05$).

Enhanced effectiveness in restoring short-term memory was consistently observed in CBI rats following both separate and combined SEM and HA administration on the 5th ($p < 0.01$) and 7th ($p < 0.05$) days of the study.

1.3. Long-term memory

Throughout the post-ischemic period, there was an increment in the number of CS and US pairings required for CAAR development over the 7 days after CR induction, indicative of pronounced long-term memory impairment ($p < 0.05$). On the 1st day of the study, the investigated index value in the CBI group of rats that received combined SEM and HA administration was 4.1 ± 0.4 , identical to the respective index in the control group ($p > 0.05$).

On the 3rd day of the study, the number of CS and US pairings required for the CAAR development during 7 days after the CR produce in the group of CBI rats treated with SEM and HA combination was 4.0 ± 0.4 , which was 2.1 times lower compared to the respective index in CBI rats without pharmacological correction and 1.8 times lower compared to CBI rats that received HA (in all cases $p < 0.01$).

Similar trends, indicating a more pronounced effectiveness in restoring long-term memory in CBI rats due to the separate administration of SEM and the combined use of SEM and HA, were observed on the 5th and 7th days of the study ($p < 0.01$ in all cases).

1.4. 'Preservation'

The absolute values of the integrated 'preservation' index, which enables the assessment of the impact of the applied pharmacological agents on the maintenance of habits ('engrams') in CBI rats, are depicted in Fig. 3. A distinct decline in the 'preservation' index is evident during the post-ischemic period, starting from the 3rd day of the study, with these changes becoming statistically significant ($p < 0.05$, Fig. 3).

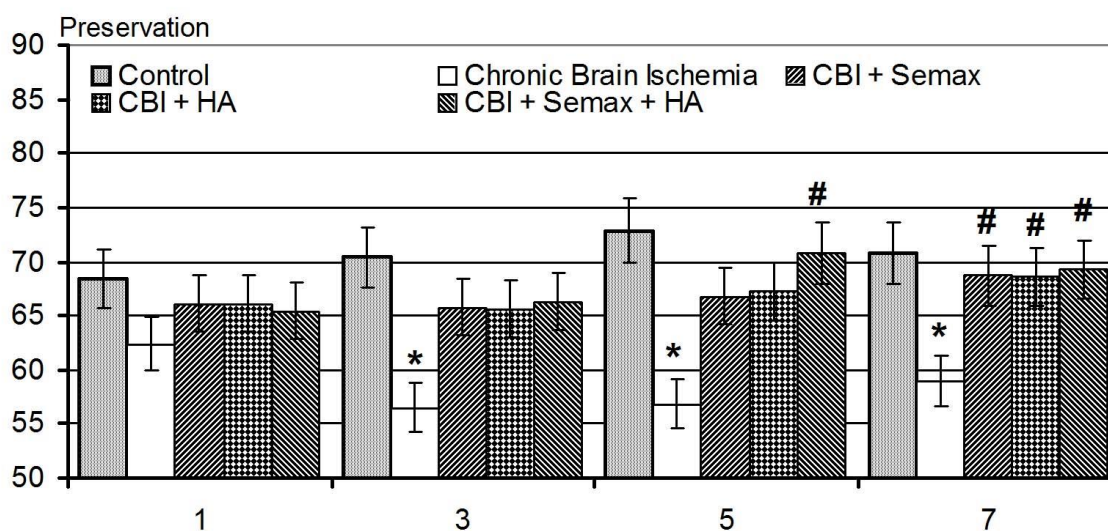


Fig. 3. Impact of separate and combined administration of Semax and hopantenic acid on the 'preservation' index in rats with CBI.

Notes. ** – $p < 0.01$ – probable discrepancies of the investigated indicators compared to those in intact rats;

– $p < 0.05$ i ## – $p < 0.01$ – probable discrepancies of the investigated indicators compared to those in rats with chronic brain ischemia without pharmacological correction (ANOVA + Newman-Keuls criterion applied in all calculations).

The values of the 'preservation' index in all experimental groups of CBI rats that received pharmacological correction during the 1st and 3rd days of the study ranged from 65.4 units to 66.3 units, showing no statistical differences compared to the corresponding control indices or data in CBI rats without treatment ($p > 0.05$).

On the 5th day of the study, the value of the investigated integrated index was 24.6% higher in the group of CBI rats that received combined administration of Semax and hopantenic acid ($p < 0.05$).

On the 7th day of the study, the values of the 'preservation' index in all experimental groups of CBI rats that received pharmacological correction ranged from 68.6 units to 69.2 units, which was higher than in CBI rats without treatment ($p < 0.05$).

2. Cognitive Dysfunction Correction Study in CBI Rats using Conditioned Food Reflex Test

2.1. Training

Within 24 hrs after bilateral occlusion of the carotid arteries, the rats were immobilized and remained in the central part of the radial maze (Table 2).

Attempts to enter the maze arms until successful food localization during training emerged on the 3rd day of the post-ischemic period. In CBI rats with combined SEM and HA administration, this indicator was 0.7 ± 0.2 , which was higher compared to the respective indices in the group of CBI rats without pharmacological correction ($p < 0.05$), as well as in CBI rats receiving separate administration of Semax ($p < 0.05$) and hopantenic acid ($p < 0.01$).

On the 5th and 7th days of the study, the number of attempts to enter the maze arms until successful food localization significantly exceeded the respective indices in CBI rats without pharmacological correction ($p < 0.05$).

2.2. Maintenance of Conditioned Reflex

One hour after the development of the food reflex, CBI rats were unable to enter the radial maze arms for food localization due to their immobilization.

On the 3rd day of the study, the value of the investigated index in CBI rats receiving pharmacological correction was higher than in those without pharmacological correction ($p < 0.05$). The number of attempts to enter the maze arms until successful food localization during this period in CBI rats receiving combined SEM and HA administration was 0.8 ± 0.1 , which was higher compared to the respective indices in those without pharmacological correction ($p < 0.05$), as well as in CBI rats receiving separate administration of hopantenic acid ($p < 0.01$).

Up to the 7th day of the study, the investigated parameter significantly exceeded the corresponding ones in CBI rats without pharmacological correction ($p < 0.05$).

Table 2

Impact of Semax and Hopantenic Acid separate and combined administration on conditioned-reflex activity processes in rats with CBI in the 8-arm radial maze test

Animal Groups	Number of attempts to enter the maze arms until successful food localization		
	Training	Conditioned reflex maintenance	Conditioned reflex extinction
<i>1st day</i>			
1 Group – Control, n=7	6.7±0.6	1.0±0.1	1.0±0.1
2 Group – CBI, n=12	0***	0***	0***
3 Group – CBI + SEM, n=12	0***	0***	0***
4 Group - CBI + HA, n=12	0***	0***	0***
5 Group – CBI + SEM + HA, n=12	0***	0***	0***
<i>3rd day</i>			
1 Group – Control, n=7	3.8±0.4	1.0±0.1	2.1±0.2
2 Group – CBI, n=9	0***	0***	0***
3 Group – CBI + SEM, n=10	0.4±0.2***#	0.6±0.1#	0.4±0.1#
4 Group - CBI + HA, n=9	0	0.4±0.1#	0.4±0.1#
5 Group – CBI + SEM + HA, n=10	0.7±0.2***# @@	0.8±0.1# @@	0.6±0.1#
<i>5th day</i>			
1 Group – Control, n=7	1.2±0.2	1.0±0.1	3.3±0.2
2 Group – CBI, n=8	0.4±0.2**	0.4±0.2*	0***
3 Group – CBI + SEM, n=9	0.8±0.1#	0.9±0.1#	0.3±0.1#
4 Group - CBI + HA, n=9	0.7±0.2	0.7±0.1#	0.3±0.1#
5 Group – CBI + SEM + HA, n=10	1.0±0.2#	1.0±0.1#	0.5±0.1#
<i>7th day</i>			
1 Group – Control, n=7	1.0±0.1	1.0±0.1	4.1±0.3
2 Group – CBI, n=8	0.4±0.2**	0.4±0.1*	0***
3 Group – CBI + SEM, n=9	1.0±0.1#	1.0±0.1#	0.3±0.1#
4 Group - CBI + HA, n=9	0.8±0.1#	0.8±0.1#	0.3±0.1#
5 Group – CBI + SEM + HA, n=10	1.0±0.1#	1.0±0.1#	0.5±0.1#

Designations. Reduction in the number of rats in groups occurred due to their demise.

Notes. * - $p < 0.05$ and ** - $p < 0.01$ – probable discrepancies in the investigated indicators compared to the data in the control group;

- $p < 0.05$ and ## - $p < 0.01$ – probable discrepancies in the investigated indicators compared to the data in CBI rats without pharmacological correction;

@ - $p < 0.05$ – probable discrepancies in the investigated indicators compared to the data in CBI rats treated with Semax and/or HA (Kruskal-Wallis test applied for all calculations)

2.3. Extinction of Conditioned Reflex

To investigate the extinction resistance of the conditioned reflex in animals with a previously developed food-conditioned reflex in the test of an 8-arm radial elevated maze, no food reinforcement was provided, and the dynamics of mnemonic functions were examined during the post-ischemic period.

Within 1 hour after inducing CBI, the rats were immobilized. Due to the inability to train and monitor them for the preservation of the conditioned reflex at that moment, no attempts to resist the extinction of this reflex were made.

On the 3rd day of the study, rats with CBI without pharmacological correction were also immobilized. The CBI rats administered SEM and HA separately and in combination entered only one arm of the radial maze due to the absence of food pellets in the previously developed skill ($p<0.05$).

On the 5th and 7th days of the study, the number of attempts to enter the arms of the maze in the absence of food pellets exceeded the corresponding indicator in the CBI rats without pharmacological correction ($p<0.05$).

Discussion

Thus, the obtained data indicate that cognitive impairments develop in rats during the post-ischemic period. This is confirmed by data on the deterioration of the learning process and the significant reduction in short- and long-term memory during the CBI period. In our research, an amnesic effect was found in the dynamics of CBI and was verified by inhibiting the formation of conditioned reflexes as well as by impairing short- and long-term memory processes in CAAR and food CR tests. The deterioration of the relative 'preservation' index in the CAAR test also reflects negative neuropathophysiological processes ongoing in rats with CBI.

The obtained data are consistent with the presence of cognitive disorders in ischemic stroke and are also reflected in the clinical presentation of other cerebrovascular diseases [1, 2, 9, 16, 22, 23]. They are somewhat consistent with and explained by the previously acquired consequences of adynamia and hypodynamia in rats after bilateral occlusion of the carotid arteries [5]. Thus, with the development of cerebral ischemia, particularly during its dynamics in experimental and clinical conditions, in addition to the death of some biological organisms, a pronounced reduction and/or deficiency of motor and sensory functions are noted [1, 13], leading to a significant neurological deficit characterized by pronounced clinical manifestation [17, 23]. We believe that motor disorders and adynamia in rats with CBI significantly determine the severity of the learning process and memory function, as well as the preservation of memory engrams and resistance to their disappearance.

A significant and important result obtained is the fact of the restoration of the investigated mnemonic parameters in the case of combined SEM and HA administration. The restoration of cognitive functions was recorded in the majority of cases, starting from the 3rd day of the study and lasting until the end of the observation period, which is consistent with

data on the restoration of motor and vestibular dysfunctions during the post-ischemic period [1, 5, 14].

In an attempt to assess the neurophysiological mechanisms of learning and memory processes studied, it should be recalled that the selected models for studying cognitive functions in animals allowed us to evaluate visual, spatial, referential, and working memory types [20]. Thus, in the vast majority of cases, pharmacological correction normalized the learning process and short-term memory, which includes visual, spatial, and referential memory types. The efforts to normalize long-term memory processes were less effective, as these processes are more complex in terms of neurophysiological mechanisms, are more prone to alteration under CBI conditions, and, under the chosen study conditions, depended significantly on the motor activity of animals, which was reduced [1, 5, 14, 29].

A convincing result of this part of the experimental research is the observed effect of alleviating cognitive disorders in rats with CBI due to the impact of SEM and HA combined administration. Previously, we already hypothesized that a well-designed and adequate pharmacological correction of chronic ischemic syndrome, causing a general neuroprotective effect, should also eliminate registered behavioral disorders under modeled artificial conditions [29]. The results of this block of studies reliably demonstrated that in addition to the overall neuroprotective effect, which was expressed in reduced mortality in CBI rats, the separate and combined administration of SEM and HA contributed to the normalization of mnemonic functions.

We consider the most effective anti-ischemic therapy scheme for cognitive disorders in experimental CBI to be the simultaneous administration of Semax and HA. Following this, the next in line is the intranasal Semax administration, which, in the majority of cases, also proved to be effective as early as the 3rd day of the study. The third in terms of nootropic anti-ischemic effectiveness is the administration of HA, which we observed on the 7th day of the study. The more pronounced effectiveness of the combined SEM and HA administration is confirmed by the earlier development of restorative cognitive effects and their prevalence compared to the efficacy of their separate administration.

The obtained array of factual data confirms the effectiveness of intranasal Semax administration within the study conditions. In this case, due to the fastest penetration into the brain, elevation of effective therapeutic concentration, contact with ischemically affected neurons, and the absence of the need to cross the blood-brain barrier, the goals were achieved, which otherwise would result in a decrease in the speed and effectiveness of the therapy [1, 10-12, 22]. From the perspective of secondary neuroprotection, the speed of ensuring the

neuroprotective effect and its effectiveness are the two most crucial requirements for achieving a positive therapeutic outcome. The analysis of the obtained data indicates a correlation in the majority of cases between the anti-ischemic effects provided by intranasal Semax administration and the combined SEM and HA administration. Our data in this context coincide with the results that demonstrated the effectiveness of intranasal administration of pharmacological compounds in ischemic and traumatic brain injuries [1, 4, 5, 14, 25, 26]. With this in mind, we consider the obtained data as additional experimental rationale for the feasibility of clinically testing the effects of pharmacological compounds capable of restoring learning and memory processes in patients with CBI.

It is essential to delve into the mechanisms underlying the protective effects of the applied original pharmacological complex in CBI. Semax, as the pioneer drug in the neuropeptide group, elicits neuroprotective, neurometabolic, nootropic, and anti-asthenic effects. It stimulates the synthesis of crucial neurotrophic factors in the brain, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), while also impeding primary and delayed neuronal death [3]. The effectiveness of Semax has been demonstrated in treating cerebrovascular diseases, preventing them, and addressing cognitive disorders [15].

The composition of hopantenic acid includes gamma-aminobutyric acid, which is incorporated into the molecule of D-pantothenic acid (vitamin B5) instead of a fragment of beta-alanine, enhancing its penetration through biological membranes into the brain. This pharmacological compound exhibits anti-asthenic, activating, nootropic, and vegetotropic effects [3]. Another study highlights the efficacy of hopantenic acid [8] in combat veterans with chronic pain syndrome and post-traumatic stress disorder. It manifested in a positive impact on memory status, reduced anxiety, and diminished emotional strain. Analyzing these findings, the mechanisms through which the chosen drugs in our study exert anti-ischemic effects become evident.

Hence, grounded on the mechanisms of the restorative effects of each applied pharmacological agent, we deem the pathogenetically substantiated scheme for pharmacological correction of cognitive disorders in CBI, developed and tested by us, as well-justified. Its efficacy is attributed to the activation of sanogenetic mechanisms.

Consequently, the demonstrated effectiveness of the developed pathogenetically substantiated complex for correcting mnemonic disorders during the post-ischemic period indicates the emergence of a nootropic anti-ischemic effect and underscores the fundamental potential of enhancing treatment effectiveness for patients with chronic brain ischemia through intranasal Semax administration, either alone or in combination with HA. Hence, we

regard the acquired data as a foundational basis for the prospective clinical exploration of the nootropic effects of combined Semax and HA administration in cases of chronic brain ischemia.

Conclusions

1. Cognitive impairments manifest early in rats with chronic cerebral ischemia, evident through disrupted learning processes and compromised short-term and long-term memory functions, assessed via conditioned active avoidance and conditioned feeding reflexes.

2. The separate and combined administration of Semax and HA contributes to the restoration of learning processes and the enhancement of mnemonic functions in rats afflicted with chronic cerebral ischemia.

3. The most profound nootropic effect in rats with chronic cerebral ischemia was observed with the combined administration of Semax and HA, effective from the 3rd day of the study. Semax alone exhibited anti-ischemic efficacy starting from the 3rd day, while HA's neuroprotective effect emerged from the 7th day.

4. The anti-ischemic effectiveness of Semax was optimized through intranasal administration, facilitating its swift impact on neurons under ischemic conditions.

5. The obtained data serve as an empirical rationale for considering clinical trials to explore the nootropic effects of Semax and HA in combination for cases of chronic cerebral ischemia.

6. Comprehensive pharmacological correction of cognitive disorders in chronic cerebral ischemia is well-grounded in its pathogenesis, with the aim of activating sanogenetic mechanisms.

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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.