DOI 10.1007/s11055-023-01347-2
Neuroscience and Behavioral Physiology, Vol. 52, No. 8, October, 2022

Changes in Psychoemotional State in Response to Neuroactive Amino Acid Derivatives in Rats after Chronic Alcohol Intoxication

L. E. Borodkina, Yu. A. Smolnyakova, E. A. Muzyko, and I. N. Tyurenkov

Translated from Zhurnal Nevrologii i Psikhiatrii imeni S. S. Korsakova, Vol. 122, No. 4, Iss. 1, pp. 112–116, April, 2022. Original article submitted September 17, 2021. Accepted January 11, 2022.

Objectives. To study the effects of neuroactive amino acid derivatives glufimet and mefargin on the psychoemotional state of rats after chronic alcohol intoxication (CAI). **Materials and methods.** Studies were carried out on 10-month-old female Wistar rats with a model of CAI produced by replacing drinking water with 10% ethyl alcohol solution sweetened with sucrose (50 g/liter) for six months. At the end of alcoholization, the animals (age 16 months) were divided into groups: group 1 ($n = 15$) was the intact group, i.e., rats without CAI; group 2 ($n = 14$) were controls, i.e., females after CAI; groups 3 ($n = 11$), 4 ($n = 14$), 5 ($n = 11$), and 6 ($n = 10$) were females which after developing CAI were treated with GABA derivative mefargin (25 mg/kg), glutamic acid derivative glufimet (29 mg/kg), or reference agents phenotropil (25 mg/kg) or heptral (100 mg/kg), respectively. Substances were administered to rats after six months of alcoholization i.p. once a day for 14 days; the intact and control groups received saline. The psychoemotional state of the animals was assessed after treatment using the open field, elevated plus maze, marble-burying, and Porsolt forced swimming tests. **Results.** Animals of the control group displayed increased anxiety, as evidenced by the greater number of boluses in the open field test compared with the intact group ($p < 0.05$) (3.43 ± ± 0.56 vs. 1.47 ± 0.39), shorter durations of stays in the open arms (26.07 ± 3.47 versus 68.67 ± 10.08), and fewer hangings from them (3.07 ± 0.25 versus 6.67 ± 0.79) in the elevated plus maze test. Rats buried more marbles after CAI (8.79 ± 1.15 versus 2.73 ± 0.71, $p < 0.05$), indicating compulsive behavior. In addition, control females had a depressed state, as evidenced by a long period of immobility in the Porsolt forced swimming test (2.36 ± 0.41 versus 0.87 ± 0.31, $p < 0.05$). **Conclusions.** Mefargin, glufimet, phenotropil, and heptral contributed to limiting anxiety and signs of obsessive-compulsive disorder in females exposed to alcohol ($p < 0.05$). An antidepressant effect was seen in animals treated with phenotropil and heptral after CAI ($p < 0.05$).

Keywords: chronic alcohol intoxication, neuroactive amino acid derivatives, mefargin, glufimet, psychoemotional state.

Data from the Global Information System on Alcohol and Health (GISAH), developed by the World Health Organization indicate that the average amount of alcohol consumed in the world in 2018 was 6.2 liters for every person over 15 years of age. It is well known that chronic alcoholism and withdrawal symptoms are associated with a number of psychoemotional disorders [1, 2]. Anxiety and depressive reactions are often noted on withdrawal of ethanol, and the likelihood of developing these conditions is known to be significantly higher in women than in men [3]. Anxiety and depression occurring in people with alcohol dependence have been shown to be more commonly associated with the effects of ethanol on the central nervous system rather than diseases preceding alcoholism [4]. Prolonged use of ethanol gradually switches a person's behavior from controlled to compulsive [5], which is also one of the risk factors for recurrence of alcohol intake after courses of treatment for addiction. In this light, the issue of ethanol abuse is an important medical and social problem and the

Volgograd State Medical University, Russian Ministry of Heath, Volgograd, Russia; e-mail: muzyko.elena@mail.ru.

search for highly active and safe substances correcting the complications of chronic alcohol intoxication (CAI) remains relevant.

As the occurrence of psychoemotional disorders in alcoholism is now demonstrated to be caused by cell damage in a variety of brain structures, changes in the functioning of neurotransmitter systems, impaired metabolism of nervous tissue cells, and mitochondrial dysfunction, neuroactive amino acid derivatives are promising drugs for correcting such abnormalities. Previous studies have shown that in stress conditions, the glutamic acid derivative glufimet improves the respiratory function of brain mitochondria and has a marked antioxidant effect [6]. The composition of the GABA derivative mefargin includes 4-amino-3-phenylbutanoic acid methyl ester hydrochloride (mefebut), which has a neuroprotective effect and corrects neuropsychiatric disorders in rats [7], as well as L-arginine, which is regarded as an indispensable component of the complex therapy of various forms of cerebrovascular lesions [8].

The aim of the present work was to was to study the effects of neuroactive amino acid derivatives mefargin and glufimet on the psychoemotional state of rats after CAI.

Material and Methods. Studies were performed using 75 white female Wistar rats aged 10 months and weighing 280–320 g. Rats were obtained from the laboratory animal supplier Stolbovaya (Moscow District) and were kept in a 12-h light regime at a temperature of 21–22°C, humidity 40–55%. CAI was modeled by replacing drinking water with 10% ethanol solution with sucrose (50 g/liter) for six months [9]. Rats were 16 months old when alcoholization ended. Groups were formed at this time point.

Group 1 (n = 15) consisted of intact animals without CAI and given i.p. physiological saline daily for 14 days; group 2 (n = 14) were controls and were given injections of 0.9% solution sodium chloride using the same protocol; groups 3, (n = 11), 4 (n = 14), 5 (n = 11), and 6 (n = 10) were the study groups and included females treated after CAI with mefargin (methyl ester of 4-amino-3-phenylbutanoic acid hydrochloride:L-arginine, 1:1, substance synthesized at the Herzen Russian State Pedagogical University, Russia) at a dose of 25 mg/kg, glufimet (β-phenyl-hydrochloride glutamic acid dimethyl ester; substance synthesized at the Herzen Russian State Pedagogical University, Russia) at a dose of 29 mg/kg, or reference agents phenotropil 25 mg/kg (4-phenylpiracetam; substance synthesized at the Herzen Russian State Pedagogical University, Russia), or heptral 100 mg/kg (S-adenosyl-L-methionine-1,4-butane disulfonate; Famar L'Aigle, France). Phenotropil and heptral were selected as reference agents as they are used in clinical practice to correct withdrawal symptoms, alcohol intoxication, neurosis, depression, and various encephalopathies.

After treatment was complete, the open field, elevated plus maze, marble-burying, and Porsolt forced swimming tests were run to study the psychoemotional state of the animals. The open field test was carried out for 3 min in a round open field test apparatus for rats (diameter 97 cm, NPK Open Science, Russia). The numbers of sectors crossed, peeps into holes, rearings without support and with support, and boluses were recorded. The elevated plus maze test was run in an apparatus consisting of a cross-shaped platform with two open (OA) and two opaque closed (CA) arms 50 cm long and 14 cm wide (NPK Open Science, Russia). The latent period (LP) of departure from the central zone (CZ), the numbers of entries into, and the times spent by rats in the OA and CA, the number of rearings and hangings in the OA, and the duration of the animal's stay in the CZ were recorded for 3 min. In the marble-burying test, rats were placed for 30 minutes in a cage of size 42 × 26 × 15 cm with a 5-cm layer of densely packed sawdust. Glass marbles were then laid out in the cage as a pattern of four rows of five marbles, and the rat was left in the cage for 60 min. The number of buried marbles (submerged in the litter by more than 2/3) was then counted. The Porsolt forced swimming test was run by placing rats in transparent plastic cylinders (height 45 cm, diameter 20 cm, NPK open Science, Russia) filled with water to 10 cm from the top (temperature 25 ± 2°C); the LP of immobility and the durations of immobility and active swimming were recorded for 3 min.

Statistical processing of study data was run in Statistica v.12.5 (StatSoft Inc., USA). Paired comparisons were run using Student's test and the Mann–Whitney test, while multiple comparisons used the Newman–Keuls and Kruskal–Wallis tests with Dunn's post-test. Between-group differences were regarded as statistically significant at $p < 0.05$.

All experimental procedures with animals were performed neuron compliance with the requirements of the interstate standard of the Russian Federation GOST R 33044-2014, *Principles of Good Laboratory Practice*, and the Directives of the European Parliament and the Council of the European Union 2010/63/EU on the protection of animals used for scientific purposes. The study protocol was approved by the Regional Independent Ethics Committee of the Volgograd Region: Protocol No. 2095–2019 of January 25, 2019.

Results and Discussion. After CAI, rats in the control group showed decreases in exploratory, horizontal, and vertical motor activity, as evidenced by smaller numbers of peeps into holes, sectors crossed, and rearings in the open field test as compared with the intact group. The number of boluses in females subjected to alcoholization was statistically significantly higher than that in animals without CAI, indicating an elevated level of anxiety in the former. Rats treated with mefargin, glufimet, and phenotropil after alcoholization made significantly more rearings than animals of the control group. At the same time, smaller numbers of boluses were seen in females treated with glufimet, phenotropil, and heptral (Table 1).

Data obtained in the elevated plus maze test evidenced high levels of anxiety in rats after CAI – females of the control group spent significantly less time in the OA, made fewer entries into and hangings from the OA, visited the CA

TABLE 1. Indicators of Behavioral Activity in the Open Field Test in Female Rats by Group (M ± m)

Parameter	Intact (n = 15)	Control (n = 14)	Mefargin (n = 11)	Glufimet (n = 14)	Phenotropil (n = 11)	Heptral (n = 10)
Sectors crossed	36.93 ± 2.23	29.21 ± 1.76*	33.91 ± 1.16	35.43 ± 2.88	33.09 ± 1.85	24.80 ± 4.02$^{@}$
Rearings	10.13 ± 0.84	7.5410.82*	10.10 ± 0.31$^{\#}$	11.42 ± 1.21$^{\#}$	13.33 ± 2.01$^{\#}$	7.00 ± 0.93$^{\&@+}$
Peeps into holes	6.93 ± 0.60	6.29 ± 0.55	6.82 ± 0.93	9.93 ± 0.85$^{\#@<}$	11.18 ± 3.84$^{\#\&<}$	5.70 ± 0.73
Boluses	1.47 ± 0.39	3.43 ± 0.56$^{\wedge}$	3.09 ± 0.84	1.36 ± 0.13$^{\$}$	0.64 ± 0.31$^{\$}$	1.10 ± 0.10$^{\$}$

Here and in Tables 2–4: statistically significant differences compared with: *intact group (Student's t test); $^{\wedge}$intact group (Mann–Whitney U-test); $^{\#}$control group (Newman–Keuls test); $^{\&}$rats after CAI (chronic alcohol intoxication) treated with mefargin (Newman–Keuls test); $^{@}$rats after CAI treated with glufimet (Newman–Keuls test); $^{+}$rats after CAI treated with phenotropil (Newman–Keuls test); $^{<}$rats after CAI treated with heptral (Newman–Keuls test); $^{\$}$control group (Kruskal–Wallis test with Dunn's pos$t$ test), $p < 0.05$.

TABLE 2. Parameters of Behavioral Activity in the Elevated Plus Maze Test in Female Rats by Group (M ± m)

Parameter	Intact (n = 15)	Control (n = 14)	Mefargin (n = 11)	Glufimet (n = 14)	Phenotropil (n = 11)	Heptral (n = 10)
LP of exit from CZ, sec	1.73 ± 0.28	2.07 ± 0.46	1.91 ± 0.39	2.07 ± 0.49	4.55 ± 1.45	4.00 ± 2.04
Entries to OA	2.93 ± 0.30	1.93 ± 0.16$^{\wedge}$	3.73 ± 0.70$^{\$}$	2 .36 ± 0.25	3.18 ± 0.30$^{\$}$	2.90 ± 0.35
Time in OA, sec	68.67 ± 10.08	26.07 ± 3.47*	53.09 ± 7.30$^{\#}$	48.86 ± 6.57$^{\#}$	57.45 ± 11.41$^{\#}$	57.30 ± 9.60$^{\#}$
Hangings in OA	6.67 ± 0.79	3.07 ± 0, 25*	6.18 ± 0.64$^{\#}$	5.64 ± 0.66$^{\#}$	7.64 ± 0.96$^{\#}$	5.20 ± 0.80$^{\#}$
Rearings in OA	1.00 ± 0.24	0.29 ± 0.13$^{\wedge}$	0.64 ± 0.20	0.14 ± 0.10	1.09 ± 0.31	0.50 ± 0.22
Entries into CA	2.73 ± 0.27	3.57 ± 0.34*	3.27 ± 0.56	3.29 ± 0.41	3.27 ± 0 .52	3.30 ± 0.52
Time in CA, sec	96.87 ± 11.49	131.29 ± 4.84*	108.18 ± 8.82	114.21 ± 7.62	96.257 ± 9.93$^{\#}$	86.00 ± 10.12$^{\#}$
Time in the CZ, sec	12.73 ± 5.20	20.57 ± 5.04	16.82 ± 3.59	14.86 ± 5.36	21.73 ± 4.97	32.70 ± 8.73

TABLE 3. Parameters of Behavioral Activity in the Marble Burying Test in Female Rats by Group (M ± m)

Parameter	Intact (n = 15)	Control (n = 14)	Mefargin (n = 11)	Glufimet (n = 14)	Phenotropil (n = 11)	Heptral (n = 10)
Number of marbles buried	2.73 ± 0.71	8.79 ± 1.15*	3.82 ± 0.67#	2.93 ± 0.82#	2.00 ± 0.59#	2.50 ± 0.48#

TABLE 4. Parameters of Behavioral Activity in the Porsolt Forced Swimming Test in Female Rats by Group (M ± m)

Parameter	Intact (n = 15)	Control (n = 14)	Mefargin (n = 11)	Glufimet (n = 14)	Phenotropil (n = 11)	Heptral (n = 10)
LP of motor activity, sec	1.00	1.00	1.00	1.00	1.27 ± 0.19	1.00
Duration of immobility, sec	0.87 ± 0.31	2.36 ± 0.41$^{\wedge}$	1.45 ± 0.47	1.64 ± 0.37	0.73 ± 0.14$^{\$}$	0.78 ± 0.15$^{\$}$
Duration of active swimming, sec	178.13 ± 0.31	176.64 ± 0.13$^{\wedge}$	177.55 ± 0.47	177.36 ± 0.37	178.00 ± 0.40	178.22 ± 0.22

of the maze more often, and stayed in them longer, as compared with intact rats. Rats injected with mefargin, glufimet, phenotropil and heptral after CAI showed less anxiety than the control group, as indicated by longer stays in the OA and larger numbers of hangings from them (Table 2).

In the marble-burying test, animals of the control group buried more marbles than intact rats, evidencing compulsive behavior in the former. Females injected with test substances buried statistically significantly fewer marbles than animals of the control group (Table 3).

In the Porsolt forced swimming test, female rats injected with saline after CAI displayed longer periods of immobility and the duration of active swimming was statistically significantly shorter than that in the intact group, indicating depressive behavior in post-alcoholization animals. Females treated with phenotropil and heptral showed decreases in the duration of immobility (Table 4).

Thus, this study showed that female rats subjected to CAI displayed signs of increased anxiety, depression, and compulsive behavior.

The development of these psychoemotional disorders in CAI and during the withdrawal period may be due to damage to and changes in the functioning of various brain structures – the prefrontal and insular cortex, the amygda-

la, and the hippocampus, which are involved in regulating emotional behavior [2, 5, 10, 11]. Neuroinflammation, intensification of apoptotic processes, and oxidative and nitrosative stress play leading roles in alcohol-induced cell death in the nervous system [12]. A study reported by Arzua et al. [13] found that in vitro ethanol caused apoptosis of neurons and astrocytes, while ultrastructural changes such as destruction of mitochondrial cristae and disorganization of the cytoskeleton were seen in cells, apparently due to increased formation of reactive oxygen species and decreased activity of antioxidant defense enzymes [14]. CAI has been shown to be associated not only with abnormal mitochondrial morphology, but also with suppression of their respiratory capacity in the medial prefrontal cortex of the mouse brain [15]. Similar changes have been noted in the hippocampus [16].

Improvements in the psychoemotional state of animals treated with mefargin, glufimet, phenotropil, and heptral after CAI are probably due to the polytropic effects of these compounds. Mefargin contains mefebut and L-arginine. The former improves cerebral circulation, eliminates disturbances in locomotor and exploratory behavior, and reduces the severity of cognitive deficits and seizures [7], while L-arginine is a donor of NO, which is involved in interneuronal regulation [17]. Glufimet promotes the coupling of tissue respiration and oxidative phosphorylation in brain mitochondria in stressed rats, limits lipid peroxidation, and increases the activity of antioxidant enzymes [6]. Published data indicate that phenotropil has activating, anxiolytic, and antidepressant effects, reduces the manifestations of asthenia and intellectual-mnestic disorders, and increases the resistance of tissues to hypoxia and toxic actions [18]. Heptral has a marked neuroprotective effect in CAI, which may be associated with restriction of nitrosative stress, decreased expression of nitric oxide synthase, and overproduction of peroxynitrite in brain mitochondria [19].

Conclusions. Thus, signs of psychoemotional disorders were seen in female rats after CAI, manifest as an anxious-depressive state and compulsive behavior. The GABA and glutamic acid derivatives mefargin and glufimet, respectively, as well as the reference drugs phenotropil and heptral, had anxiolytic and anticompulsive actions. Antidepressant effects were noted in animals treated with phenotropil and heptral after CAI.

The authors declare no conflict of interest.

REFERENCES

1. R. K. McHugh and R. D. Weiss, "Alcohol use disorder and depressive disorders," *Alcohol Research*, **40**, No. 1, arcr.v40.1.01 (2019), https://doi.org/10.35946/arcr.v40.1.01.
2. E. A. Flook, J. R. Luchsinger, M. M. Silveri, et al., "Anxiety during abstinence from alcohol: A systematic review of rodent and human evidence for the anterior insula's role in the abstinence network," *Addict. Biol.*, **26**, No. 2, e12861 (2021), https://doi.org/10.1111/adb.12861.
3. G. Petit, O. Luminet, M. Cordovil de Sousa Uva, et al., "Gender differences in affects and craving in alcohol-dependence: A study during alcohol detoxification," *Alcohol Clin. Exp. Res.*, **41**, No. 2, 421–431 (2017), https://doi.org/10.1111/acer.13292.
4. C. Gallagher, Z. Radmall, C. O'Gara, and T. Burke, "Anxiety and depression among patients with alcohol dependence: co-morbid or substance-related problems?" *Ir. J. Psychol. Med.*, **35**, No. 2, 121–126 (2018), https://doi.org/10.1017/ipm.2017.25.
5. V. Vengeliene, E. Celerier, L. Chaskiel, et al., "Compulsive alcohol drinking in rodents," *Addict. Biol.*, **14**, No. 4, 384–396 (2009), https://doi.org/10.1111/j.1369-1600.2009.00177.x.
6. I. N. Tyurenkov, T. A. Popova, V. N. Perfilova, et al., "Protective effects of a new glutamic acid derivative against stress after nNOS blockade," *Biomed. Khim.*, **63**, No. 1, 47–55 (2017), https://doi.org/10.18097/PBMC20176301047.
7. V. V. Bagmetova, I. N. Tyurenkov, L. E. Borodkina, et al., "Neuroprotective effects of the methyl ester of fenibut and its formulations with organic acids in correcting psychoneurological impairments induced by convulsive pathology," *Fundament. Issled.*, **3–1**, 22–26 (2013).
8. S. G. Burchinskii, "Angioprotection: protection of cerebral vessels in family medical practice," *Semeinaya Med.*, **5**, 57–60 (2017).
9. S. A. Kryzhanovskii, I. B. Tsorin, L. G. Kolik, et al., "A translation model of alcoholic cardiomyopathy," *Molek. Med.*, **3**, 40–47 (2015).
10. J. Yuanyuan, Z. Junyan, D. Cuola, et al., "Memantine attenuated alcohol withdrawal-induced anxiety-like behaviors through down-regulating NR1-CaMKII-ERK signaling pathway," *Neurosci. Lett.*, **686**, Supplement, 133–139 (2018), https://doi.org/10.1016/j.neulet.2018.09.006.
11. B. M. Walker, D. A. Drimmer, J. L. Walker, et al., "Effects of prolonged ethanol vapor exposure on forced swim behavior, and neuropeptide Y and corticotropin-releasing factor levels in rat brains," *Alcohol*, **44**, No. 6, 487–493 (2010), https://doi.org/10.1016/j.alcohol.2010.06.006.
12. H. Xu, H. Li, D. Liu, et al., "Chronic voluntary alcohol drinking causes anxiety-like behavior, thiamine deficiency, and brain damage of female crossed high alcohol preferring mice," *Front. Pharmacol.*, **12**, 614396 (2021), https://doi.org/10.3389/fphar.2021.614396.
13. T. Arzua, Y. Yan, C. Jiang, et al., "Modeling alcohol-induced neurotoxicity using human induced pluripotent stem cell-derived three-dimensional cerebral organoids," *Transl. Psychiatry*, **10**, No. 1, 347 (2020), https://doi.org/10.1038/s41398-020-01029-4.
14. V. D. Reddy, P. Padmavathi, G. Kavitha, et al., "Alcohol-induced oxidative/nitrosative stress alters brain mitochondrial membrane properties," *Mol. Cell. Biochem.*, **375**, No. 1–2, 39–47 (2013), https://doi.org/10.1007/s11010-012-1526-1.
15. P. Shang, D. Lindberg, P. Starski, et al., "Chronic alcohol exposure induces aberrant mitochondrial morphology and inhibits respiratory capacity in the medial prefrontal cortex of mice," *Front. Neurosci.*, **14**, 561173 (2020), https://doi.org/10.3389/fnins.2020.561173.
16. C. Tapia-Rojas, R. G. Mira, A. K. Torres, et al., "Alcohol consumption during adolescence: a link between mitochondrial damage and ethanol brain intoxication," *Birth Defects Res.*, **109**, No. 20, 1623–1639 (2017), https://doi.org/10.1002/bdr2.1172.
17. M. Cossenza, R. Socodato, C. C. Portugal, et al., "Nitric oxide in the nervous system: biochemical, developmental, and neurobiological aspects," *Vitam. Horm.*, **96**, 79–125 (2014), https://doi.org/10.1016/B978-0-12-800254-4.00005-2.
18. T. V. Potupchik, O. F. Veselova, and I. V. Gatskikh, "Pharmacotherapeutic aspects of the use of nootropics in people with alcohol dependence," *Med. Alfavit*, **2**, No. 19, 37–41 (2019), https://doi.org/10.33667/2078-5631-2019-2-19(394)-37-41.
19. I. F. Belenichev and T. V. Kucher, "The influence of thiol antioxidants on the state of nitrosating stress in the brains of rats with chronic ethanol intoxication," *Farmakol. Likars. Toksikol.*, **2**, 24–29 (2016).