

Evaluation of the Neurological Status in Experimental Acute Alcohol Intoxication

E. Y. Bonitenko, A. N. Grebenyuk*, V. A. Basharin*,
M. B. Ivanov*, and N. V. Makarova

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Approaches to evaluation of the severity of neurological disorders in animals with experimental acute severe alcohol intoxication were developed. Using the method of statistical analysis, we selected criteria, which can be used for evaluation of the severity of inhibition of the nervous system in studies of the alcoholism pathogenesis and for improvement of methods for correction of severe intoxication at the preclinical stage of drug efficiency evaluation.

Key Words: *ethanol; intoxication; neurological status; coma*

Ethanol (toxic) coma is the most severe manifestation of psychoneurological disorders in ethanol poisoning. This urgent state requires immediate therapy [2,3]. Study of the pathogenesis and improvement of treatment protocols and means for toxic coma control are priority trends in experimental and clinical medicine [5,6]. Adequate preclinical studies are of priority importance for solution of these problems. The term "toxic coma" in experimental animals is widely used in Russian and foreign publications, but there are no universal approaches to evaluation of animal status in ethanol intoxication [1,4,7].

Our aim was to select the criteria for evaluation of the severity of nervous system depression in experimental animals with acute severe ethanol intoxication.

MATERIALS AND METHODS

The study was carried out on 96 outbred male albino rats (180-220 g) from Rappolovo Breeding Center. The animals were kept in a vivarium in plastic cages, 6 per cage at 20-22°C and natural light with free access to fodder during the first half of the day. The animals received no fodder 24 h before the study.

Depression of the nervous system function of different severity (stunning-sopor-coma) was induced by intraperitoneal injection of ethanol (30% solution) in doses of 0.5-1.2 LD₅₀. The mean lethal (LD₅₀) dose for ethanol was 5.2 g/kg.

The status of animals poisoned with ethanol was evaluated 30 min, 1, 3, 6, and 8 h after injection by the following parameters ranked by the severity and expressed in points.

Animal behavior. Normal activity (grooming, eating, *etc.*), sleeping, active purposeful reactions if taken by the operator: 4 points; lying, slow movements if taken by the operator, but with a trend to avoidance, head turning: 3 points; lying on the abdomen or on the side, stray movements if taken by the operator: 2 points; lying on the abdomen or side, no movements if taken by the operator: 1 point.

Reaction to olfactory (food) stimulus (chocolate). Active reaction with movement, precise orientation to the stimulus: 3 points; poor reaction, head turning towards the stimulus: 2 points; no reaction: 1 point.

Audiomotor reaction. Manifest (avoidance reaction, stretching of the hind limbs, bending of fore limbs, spine arching): 3 points; poor (contraction of the annular ocular muscle, among other things): 2 points; no reaction: 1 point.

Exploratory reaction. Normal activity (sniffing, motor activity): 3 points; sitting, no apparent explor-

Institute of Toxicology, Federal Biomedical Agency of Russia; *S. M. Kirov Military Medical Academy, St. Petersburg, Russia. **Address for correspondence:** basharin1@mail.ru. V. A. Basharin

atory reaction: 2 points; no exploratory reaction, lying on the abdomen or side: 1 point.

Clutching to the grid reflex. Manifest (holding force increases if the rat is elevated): 3 points; reduced: 2 points; absent: 1 point.

Turning reflex. Retained: 4 points; delayed: 3 points; no turning, only attempts at: 2 points; no reflex: 1 point.

Muscle tone. Normal tone (elastic tone of the abdominal wall, moderate tone of the limbs): 3 points; dystonia (reduced or elevated tone): 2 points; atonia: 1 point.

Testing of equilibrium on declining surface. Animal rapidly turns towards the upper part of the platform or explores the platform: 4 points; slowly turns, sits, or lies, stray purposeful movements: 3 points; no turning, presumably dyscoordinated movements: 2 points; no movements, lateral posture: 1 point.

Testing of equilibrium on a horizontal rod (no rotation). The animal keeps sitting during more than 30 sec: 3 points; animal is clumsy and falls soon (less than 30 sec): 2 points; cannot fulfill the test (falls), cannot be put onto the rod: 1 point.

Coordination disorders (ataxia). None: 3 points; ataxia: 2 points; impossible to test (no movements, lateral posture): 1 point.

Ammonium test. Pronounced avoidance reaction to the stimulus: 3 points; poor reaction, sneezing reflex retained: 2 points; no reaction: 1 point.

Tactile and painful sensitivity. Reacts to a single exposure by aggression or avoidance: 4 points; weak reaction by crawling or turning: 3 points; nonlocalized response, including that by the abdominal wall contraction: 2 points; no reaction: 1 point.

Hind limb flexion reflex. Rapid flexion of the foot, retained for a short time: 4 points; slow flexion of the foot, retained for a long time: 3 points; no foot flexion after solitary stimulation, nonlocalized response by abdominal wall contraction: 2 points; no reaction even after repeated stimulation: 1 point.

Pupil status. Normal: 2 points; myosis or mydriasis: 1 point.

Pupil reflex (pupil reaction to light). Pronounced: 3 points; weak: 2 points; no reaction: 1 point.

Mydriasis and absence of pupil reflex. No mydriasis concomitant with failure of pupil reaction: 2 points; dilated pupil not reacting to light: 1 point.

Corneal reflex. Manifest: 3 points; poor: 2 points; no reflex: 1 point.

Pharyngeal reflex. Manifest: 3 points; poor: 2 points; no reflex: 1 point.

In addition, some parameters of autonomic functions were studied in experimental animals: status of the respiratory system, heart work, and thermoregulation system. The respiratory function was evaluated by

the respiration movement frequency and type of respiration. Electrocardiogram was recorded on a Poly-Spectrum-8B device using standard leads. The animals were placed into special plastic boxes. Electrodes (stainless steel) were inserted subcutaneously. Rectal temperature was measured with TPME-1 device.

The results were statistically processed using Statistica 6.0 software.

RESULTS

Injection of ethanol in doses of 0.5-1.2 LD₅₀ led to pronounced inhibition of the CNS function with impairment of reactions to external stimuli and progressing disorders in vital functions (respiration, circulation). Based on the analysis of published data and our experimental findings, we selected the neurological and autonomic characteristics for differentiation between the following conditions: normal status, stunning, sopor, superficial coma, deep coma, and threshold coma.

For maximally objective evaluation of the results, analysis of correlations was carried out in order to detect the relationships between the studied neurological values and status of experimental animals (Table 1). Parameter γ was selected for the most correct registration of repeating values.

A strong correlation was detected between the neurological parameters and severity of inhibition of the nervous system in animals during the studied intervals after ethanol injection. The absence of the "mydriasis and absence of pupil reflex" parameter is explained by the fact that manifestation of this sign preceded animal death. This sign is usually associated with failure of respiratory movements and 5-12 min later with cardiac activity depression. A strong correlation was detected between the severity of the status and changes in heart work. Injection of ethanol was associated with heart rate reduction. Ethanol had an appreciable impact for thermoregulation system, which manifested by reduction of the rectal temperature. Slight correlations between the degree of neurological status depression and changes in respiration can be explained by a complex effect of ethanol on the respiratory system and by significant changes in this system under the effects of ethanol metabolites.

Based on these results, a score for evaluation of neurological status was developed for the diagnosis of animal status in different periods of intoxication after ethanol injection, with calculation of the index of nervous system depression severity (IND).

The INS is calculated by the formula:

$$IND=5 \times AB+4 \times PR+3 \times PS+2 \times BR+2 \times PuR+CR,$$

where: AB is animal behavior, points; PR is pharyngeal reflex, points; PS is tactile painful sensitivity,

TABLE 1. Rank (γ) Correlations between Neurological Testing Results and Autonomic Function Parameters ($p \leq 0.05$) in Acute Ethanol Intoxication

Parameter	Period of study, h				
	0.5	1	3	6	8
Animal behavior	-1.000	-1.000	-0.985	-0.991	-1.000
Reaction to olfactory (food) stimulus	-1.000	-0.982	-1.000	-0.888	-0.953
Audiomotor reaction	-1.000	-0.941	-0.980	-0.881	-0.973
Exploratory reaction	-1.000	-1.000	-1.000	-0.965	-0.986
Clutching to the grid reflex	-1.000	-1.000	-1.000	-0.990	-0.982
Turning reflex	-1.000	-1.000	-0.942	-0.993	-0.995
Muscle tone	-0.632	-0.763	-0.974	-0.977	-0.977
Testing of equilibrium on sloping surface	-1.000	-1.000	-1.000	-0.973	-0.982
Testing of equilibrium on horizontal rod	–	-1.000	-1.000	-0.939	-1.000
Coordination disorders	-1.000	-0.981	-0.905	-0.988	-0.990
Ammonium test	-0.935	-0.960	-0.927	-0.865	-0.866
Tactile and painful sensitivity	-1.000	-0.989	-0.973	-0.964	-0.978
Hind paw flexion reflex	-0.974	-0.990	-0.875	-0.959	-0.983
Pupil status	-0.514	-0.770	-0.745	-0.736	-0.800
Pupil reflex	-0.802	-0.931	-0.890	-0.839	-0.931
Mydriasis and absence of pupil reflex	–	–	–	-0.738	–
Pupil size	0.622	0.603	0.405	0.355	0.653
Corneal reflex	-0.906	-0.956	-0.977	-0.837	-0.963
Pharyngeal reflex	-0.964	-0.938	-0.939	-0.984	-0.934
Respiration rate	-0.497	-0.325	-0.311	–	–
Heart rate	-0.618	-0.720	-0.689	-0.798	-0.830
Rectal temperature	–	-0.445	-0.666	-0.804	-0.803

Note. Parameters forming weak correlation are not shown.

points; BR is hind limb flexion reflex, points; PuR is pupil reflex, points; CR is corneal reflex, points; 5, 4, 3, and 2 are the sign significance coefficients (regression coefficients).

The regression coefficients in the formula for IND calculation were stable in different periods after ethanol injection.

Interpretation of the resultant IND values was as follows: IND>55: normal functioning of the nervous system; 46-55: stunning; 36-45: sopor; 26-35: moderate coma; 18-25: deep coma. Threshold coma is diagnosed using this rule with additional evaluation of disorders of vital functions. However, analysis of experimental findings and the use of method of calculations suggests that animals with a score of 17 and lower should be referred

to those in a state of threshold coma. These animals exhibited pronounced disorders in vital functions.

The results of use of the decisive rule with ISN estimation coincided with expert evaluation in 75% cases. The most complete coincidence was observed 0.5 and 1.0 h after ethanol injection (79 and 78%, respectively). The least number of coincidences (70%) was recorded 3 h postinjection.

Hence, experimental study enabled us to develop a scoring system for evaluation of neurological signs and create a score for evaluation of the severity of the CNS depression in ethanol intoxication. The results can be used in studies aimed at improvement of the existing and development of new methods for therapy of ethanol intoxication.

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