The Influence of energy Drinks on the emotional and behavioral Reactions of Rats

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Energy drinks (ED) have appeared in Ukraine recently, in second half of 20th century. They became popular and got preferences among teen groups. ED are one of the most dynamic segments of soft drinks market [13]. Composition of ED, available on Ukrainian market differs both by quantitative and qualitative content, but all of them have the same features: the presence of biologically active substances such as caffeine, taurin, inositol, glucuronolacton, guarana, carnitine, B group vitamins (riboflavin, niazin, vitamin B6 and B12). Manufacturers are convinced that ED composition increase attention, reaction speed, mood, productivity, ability to focus and has overall stimulation effect. Medical evidence of ED consumption is contradictory. Proponents consider that ED have continuous effect on focus of attention, memory and increase physical efficiency both in aerobic and anaerobic conditions [16, 19]. On the other side, their opponents mostly speak about side effects, such as headache, tachycardia, increased risk of brain vascular affection, mental and metabolic disorders [20, 21, 23].

There is a lack of available data in scientific literature dealing with the study of ED side effects on animal models. As the main consumers groups is youth, young animals should be used in experiment for the most precise extrapolations of experimental data on human population.

Energy drinks usage force body to work under the continuous extreme (stress) conditions, overloading nervous, cardiovascular systems, exhausting cells energetic resources. At the end of influence, those substances decrease overall human abilities to minimum and give the side clinical effects especially in a case of overdosing.

According to a some data about relative differences in motor activity, anxiety and speed of reflex response in rats, which were consuming ED in models of everyday usage, with the control group. There was also found the positive correlation of the levels of those indexes with daily ED drinking volumes [9]. The active fluctuation of endocrine and nervous systems as well as diet differences cause brain functionality changes.

All of that requires the detailed study, analysis and designing nutrition recommendations, considering preferences in usage of such products, which cause alimentary stress [20, 22]. It is also known, that youth, to compare with adults, has brighter manifestation of neurotoxic effects during the influence of biologically active psycho-stimulant substances. That is why experimental studies of ED impact on cognitive functions and behavioural reaction on young animal models are the questions of vital importance.

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Study purpose. To study the ED influence in different regimens of use together with standard diet on behavioural reactions and nervous activity of laboratory animals.

Materials and methods of study. The most spread informational tests that shows central nervous system (CNS) functional condition in a study of chemical substances effect in a laboratory animals are open field test (OF) and summation-threshold index (STI) [4, 9]. With the aim of evaluation of behavioural reactions in white rats we used OF test (sum of crossed foursquares, vertical stances, burrows, urinations, defecations and grooming) and STI, that was found by the criterion of unconditioned motion reflex reaction in response to electrical stimulation. One group took ED one time (sporadic consuming model), two times (two times per day with 3 hours gap) and constant permanent use (thirst model) per os.

Experimental study was made on white not linear young male rats with body mass of 110–130 g, after weekly quarantine. The animals were kept in standard conditions in vivarium of Danylo Halytsky LNMU, according to the rules of «good laboratory practice» (GLP), general ethic principles of animal experiments, that was setted on National congress of bioethics (Kyiv, 2000), according to the International principles of «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasburg, 1986). Animals were kept on standard vivarium diet in free access to fluid and food, in conditions that match requirements of laboratory animals keeping [3]. Animals were placed in standard metal cages in natural light and temperature 22 ± 3°C. The body weight of animals was measured daily for the control of physical conditions and volume of fluid and ED consuming. Before the measurement, rats were covered by black cap for 1 minute. In order to determine the studied parameters such a number of animals was use that provided statistically reliable results.

Experimental groups (Eg) (10 animals in one) were taking ED in different regimes: group № 1 – once per day, group № 2 – two times per day with 3 hours interval, group № 3 – instead of water for 20 days of subacute experiment. Per os fluid introduction was calculated as 1 ml/ 100 g of body mass, according to the recommendations [3, 6, 10, 13–15]. Control group (Cg) (n = 10) was drinking water in free drinking regime. Red Bull drink was chosen as a model ED, because of its leading place in a selling volume in Ukraine [13]. The motion activity and anxiety level assessment was performed in open field test. It was produced the visual counting of the number of back paws standings in a peripheral and central foursquares, grooming, number of urination and defecation during the time of experiment.

According to the methodical recommendations for the experiments on rats, the open field test was performed in special setting – square cell with 100×100 cm size, with non-transparent walls of 40 cm height. Cell floor was divided on 16 squares, each foursquare had pass-through round hole – «burrow». Open field was evenly lighted during the experiment. Each rat’s behaviour was studied during 5 minutes. The behavioural markers were: horizontal motion activity (number of crossed squares, vertical stances, burrows), emotional activity (defecation, urination) and grooming. Defecation and square crossing were both related to the emotional activity of rodents. The prognostic criteria of CNS condition are their behavioural peculiarities in open field. New surroundings were stimulating research behaviour, characterized by passive-defence reactions. Signs of them are urination and defecation as well as change of motion activity level. Assessment of vertical stances (VS) was done to research motivational component and orientation in rat behaviour. Burrow reflex (BR) was assessed as animal ability to explore open field by number of looks into burrows. BR shows cognitive activity of experimental animals. Cosmetic behaviour (CB) was assessed by counting of grooming. CB shows elements of orientation anxiety reaction in unknown (dangerous) situation. Defecation (DF), urination (UR)
are indexes of rats emotional condition, that was estimated by the number of performed urinations and defecations [2, 5].

The method of assessment summation of sub-threshold stimulus was used for studying ED influence on functional condition of CNS in laboratory rats. Threshold summation indicator was studied by special equipment, which consist of impulses electro stimulator ICE – 01. The device generates electricity with impulse duration of stimulation 0.2 s, with increased voltage with velocity of 2 V/s. It includes the organic glass vertical panel between two horizontal iron electrodes, which were wrapped up with tissue, drenched with isotonic solution of sodium chloride. The animal was placed so front paws was on positive and back on negative electrodes. Researcher slowly increased voltage to the time it causes withdrawal of one extremity. The marker of summation ability of nervous system was estimated by threshold voltage in volts [10]. The received experimental parameters were compared with the results in control group. All the statistical indexes were computed using Microsoft Excel and AtteStat software. Changes were considered as reliable at \( p < 0.05 \) [1].

**Results and discussion.** According to previous results of the consuming preferences studying, conducted by medical-students in Danylo Halytskyi Lviv National Medical University surveying, the most popular ED was Red Bull [7]. That was the main factor to choose that brand as the model ED.

New surroundings in OP test caused the development of explorer reaction (ER) of rats and caused fear, which manifested in motion along peripheral squares, and low rate of visiting central squares. The research of motion activity (MA) was assessed, along with defecations (DF) and urination (UR) numbers in open field test. Higher level of anxiety demonstrated by rats correlated with lower level of MA and higher level of DF and UR, at the same time active rats showed lower level of DF and UR, that corresponds to empiric data [11]. Testing results of experimental animals in OF tests with different level of anxiety submitted on images 1–4.

![Image 1. Testing results on 1-st day of experiment in OF test with different anxiety level](image1.png)

![Image 2. Testing results on 5-th day of experiment in OF test with different anxiety level](image2.png)

![Image 3. Testing results on 10-th day of experiment in OF test with different anxiety level](image3.png)

![Image 4. Testing results on 20-st day of experiment in OF test with different anxiety level](image4.png)
To compare with control group, experimental animals of D2 and D3 groups demonstrated higher anxiety level on 5th and 10th day of experiment with negative dynamic to 20th day and anxiety level drop at the end of experiment. It is remarkable, that at the time of experiment, anxiety level of group with low and medium base anxiety level increased, so it can be considered as gradual exhaustion of nervous system.

ED consuming level in different regimes (table 1) revealed statistically significant increasing and decreasing of rat anxiety level firstly in D3 group and D2 on 10th and 20th day of experiment. It correlates with adaptive reactions in rats due to exponential law of toxic process development, as was studied by Lazarev N.V. (1937), Kagan U.S., Traxtenberg I.Y. The received data in D1 experiment had no statistical difference with control group.

Grooming in all experimental groups correlated with motion activity. The decreasing of markers explorers burrows reaction and grooming, vertical stances and squares crossing in to the end of experiment (20th day) shows the possible nervous system exhaustion and animals fatigue development in comparison with control group. Adaptive reaction to ED consuming followed pathophysiological scenario (table 1 continuation).

STI dynamic study in experiment revealed, that continuous ED consuming in free drinking regime has undulatory value change of this index in comparison to control group (image 5).

During the experiment STI changed from increased values (+15.9 %) on 5–10th day and further decrease on 20th day of experiment (~30.11 %). It correlates with changes of animals behavioural reactions. On 20th day sporadic ED usage was effective $p < 0.05$.

In time of experiment volume of drunk fluid stayed at the same level, so it can be thought that ED usage does not form any addiction.

All the animals from experimental groups D2 and D3 survived whole experiment term, but it was observed the little delay in body weight growth. That probably points on damaging effect in 20 days ED diet and shows offset in adaptation processes, as intoxication stage model [8].
### Table 1

**Behavioural-emotional rat activity in OF test (M ± m, n = 10)**

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Crossed square number</th>
<th>Emotion activity (orientational-explorer activity)</th>
<th>Vertical stances number</th>
<th>Burrows number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>5 day</td>
<td>10 day</td>
<td>20 day</td>
</tr>
<tr>
<td>Cg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eg₁</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eg₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eg 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*according to control, *p* < 0.05.

### Table 1 (continuation)

**Behavioral-emotional rat activity in OF test (M ± m, n = 10)**

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Grooming</th>
<th>Emotional reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>5 day</td>
</tr>
<tr>
<td>Cg</td>
<td>6.1 ± 0.58</td>
<td>5.8 ± 0.54</td>
</tr>
<tr>
<td>Eg₁</td>
<td>4.5 ± 0.42</td>
<td>4.8 ± 0.51</td>
</tr>
<tr>
<td>Eg₂</td>
<td>5.0 ± 0.61</td>
<td>5.2 ± 0.61</td>
</tr>
<tr>
<td>Eg 3</td>
<td>9.3 ± 0.57*</td>
<td>9.91 ± 0.54</td>
</tr>
</tbody>
</table>

*accordingly to control, *p* < 0.05.
Thus, prolonged ED consuming (20 days), considering average volume of fluid consuming as 20 ml/day in free drinking regimes, psycho-emotional changes of rats reactions were noticeable. Energy drink usage in evaluation models of behavioural reactions, who was gradually increasing to 10th day and further decreasing on 20th day marker of explorer, orientation and motivation reactions of rats, so it can be thought as adaptive changes in case of drinking big volumes of energy drinks. Regular prolonged drinking receive in free drinking regime cause sinusous increasing and further decreasing of nervous reactions speed in summation-threshold index test, which correlates with dynamic changes in behavioural reactions. Slight affection of the dynamic of the body weight growth in laboratory animals and cause violation in adaptive processes, which can be interpreted as intoxication stage. Long term consumption of Red Bull energetic drink in free drinking regime, the same as 2 times per day, change psycho-emotional condition in rats and affect motion animal activity in open field test and in anxiety models, causing sensitization to stimulation effect of drink.

Рекомендовано до друку колисью з біотеми

REFERENCES
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Experimental study on white rats influence of energy drinks in different modes of consumption as part of the standard diet allowed to identify emotional changes of laboratory animals in the models of anxiety in daily use in the doses of 1 ml per 100 g of rat body weight to volume of fluid drunk freely drinking regime to 20 ml per day. The preliminary survey of medical students was held before, and according to that survey energy drink Red Bull was in consumer preference that is why it was chosen for a model substance. In the test of open field the quantity of visits of peripheral and central squares, looking into the hole, freezing on the periphery and acts of defecation and urination, grooming was determined, dividing rats into subgroups with high, medium and low levels of anxiety, based on an evaluation of fear and research activity manifestation by standard methods. Moving of the rat in the new environment has stimulated a research behaviour, accompanied by passive defensive reactions that are considered to be characteristic manifestation of the vegetative reactions such as defecation and a diuresis and changes in the level of physical activity. Vertical stands were informative towards research on the motivational component and orientational behaviour of rats. Reflex mink evaluated as the ability of animals to explore the open field and in the number of looking into holes. Number of surveyed holes – «hole reflex» testified cognitive activity in experimental animals. Cosmetic behaviour of rats (grooming) – was estimated by the number of times of rats body combing. Cosmetic behaviour showed elements orientational-anxiety reactions to unfamiliar (threatening) situation.
The use of energy drinks in the evaluation model behaviours characterized by a gradual increase to 10 days and a further decline in 20 day performance research, orientational and motivational reactions in rats during the consumption of large quantities of Red Bull. Regular long-term intake of energy drink in a free drinking regime caused in rats undulating growth and further decrease in speed of nervous reactions in the threshold summation, that correlate with dynamic of changes in behavioural responses, slightly affect the dynamics of weight gain in laboratory animals and leads to changes in the process of adaptation that can be interpreted as the phase of intoxication. Also long-term use of energy drink Red Bull in free mode drinking and twice a day, changed the emotional status of rats and influence the motor activity of animals in the open field test, including models of anxiety, causing sensitization to the stimulating effect of the beverage. It was found that changes in research, orientation and motivational reactions in laboratory animals indicate possible adaptive changes in the use of large amounts of energy drinks, forcing the body to work in extreme mode with not only overload but also with further exhaustion of the nervous, cardiovascular and excretory systems. It is important to study the physiological and behaviour responses in long-term regular use of energy drinks on young animals, since the extrapolation of experimental data on human population must be taken into account that young people in most cases are the contingent of consumption of energy drinks.

Key words: Energy drinks, laboratory rats, anxiety, summation-threshold index (STI), the test of «open field».