

*Human and Animal Physiology*

## Changes of Exploratory, Locomotor and Motivational-Emotional Behavior in High Immobilization “Depressive” Rats

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**ABSTRACT.** The present study investigated the character of behavioral disturbances in high immobilization “depressive” rats. Namely, changes of exploratory, locomotor and motivational-emotional behavior in the open field. Low immobilization “non-depressive” and high immobilization “depressive” rats were selected by Porsolt’s forced swim test. We show that “depressive” animals exhibit reduction of locomotor activity, significant decrease of grooming behavior, apparent reduction in the frequencies of center entrance and the time of staying in the center of open field. In other words high immobilization “depressive” rats exhibited complete loss of interest and reduction of exploratory motivation. These findings indicate that high immobilization “depressive” rats are characterized by behavioral “despair” or “refractory loss of interest” in contrast to the low immobility “non-depressive” rats. In conclusion, genetically determined natural differences in rats behavior in the Porsolt’s forced swim test manifested in the level of immobility, helplessness in the inescapable device and the speed of surrender to this situation appeared to be the significant factors that make it possible to reveal more substantial differences in the behavior between low immobilization “non-depressive” and high immobilization “depressive” rats. It was shown by us that rats with high immobilization manifest depression-like behavioral disturbances in the open field test. The ability for real assessment of the level of danger in the new extremely illuminated area is much diminished and “depressive” rats actually underestimate the surrounding environment. © 2016 Bull. Georg. Natl. Acad. Sci.

**Key words:** depressive rats, behavioral disorders, forced swim test

Unipolar depressive disorder is a heterogeneous disease, accompanied by the complex of disturbances of various physiological functions [1]. Changes in exploratory, locomotor and motivational-emotional behavior are considered among them [2-7]. Namely,

it is believed that patients with unipolar depression suffer from suppression of motivation and emotional processes and inability to experience pleasure that is called anhedonia [1,8]. However, until now the results of observations are unequivocal in this regard.

In particular, it is not wholly understandable yet, which components of motivational and emotional behavior are more vulnerable to this disease. Therefore, the study of changes in exploratory, locomotor and motivational-emotional behavior in animals, considered as an animal model for depressive-like state, is important and topical. Using animal models in researches of pathophysiology of many psycho-nervous diseases is a modern priority approach for many research institutions in worldwide, including our laboratory [3,4,6,7,9,10].

Therefore, the aim of the present work was to study the character of the changes in exploratory, locomotor and motivational-emotional behavior in “depressive” rats.

## Materials and Methods

Experiments were carried out on adult white wild rats (weight 200-250 g). Animals were subdivided into two groups (n=10 in each group): “depressive” (experimental) and non-depressive” (control). Selection was made by means of Porsolt’s forced swim test (FST) [11, 12].

**Porsolt’s test apparatus.** Porsolt’s test apparatus represents an acrylic glass cylinder filled with water from which animal can’t escape. Animals are subjected to two trials, during which they are forced to swim in the cylinder. The first trial lasts 15 minutes. Then, after 24-hours, a second trial is performed that lasts 5 minutes. The time that the animal spends in the first and second trials without making any movements, beyond those required to keep its head above water, is measured. This is the immobility time. Modern implementations of the test score swimming and climbing behaviors separately. Duration of immobility time is one of most adequate indicator for depressive-like state in rats.

**Open field test.** Changes in exploratory, motivational and emotional behavior were studied in open field test (OF) [13]. OF is an arena of 96 cm in diameter with walls (50 cm in height) to prevent rats escape. Commonly, on the arena of the field the squares are

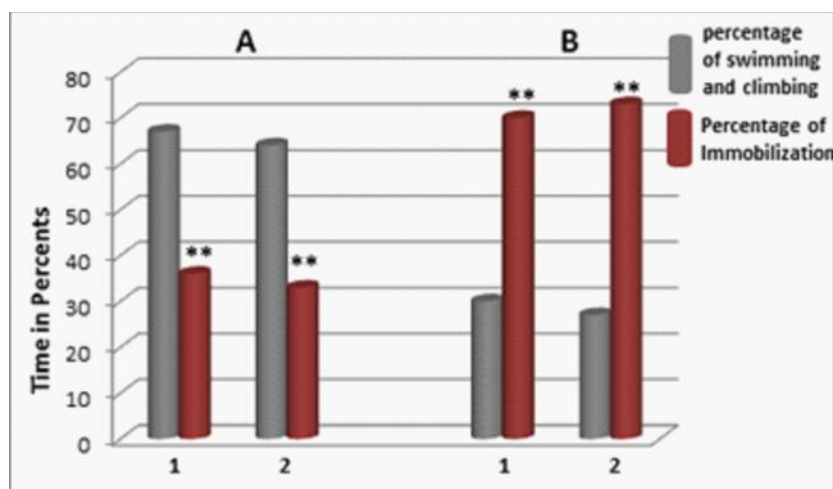
marked by white lines (each square is 12 cm ). The periphery is lighted by three light bulbs (60 watts power), while the center is lighted by one light bulb (100 watts).

Rearing and time spent moving, number of crossed squares, sniffing and had rising are used to assess the locomotor and exploratory activity of the rodents. The OF test is also often used to assess emotional behavior and anxiety by including additional measures of defecation, urination, number of entrance in the center of open field and time spent in the center of the field. At the beginning of the experiment rats are placed on the periphery of the OF and then their behavior is explored during 10 minutes. Obtained results are treated for the first 5 min, second 5 min and for the whole 10 min of exploration. Separate analysis of the data from the different periods of observation might reveal the level of habituation to the OF and possible changes of habituation in “depressive” rats.

All procedures were conducted with the NJH Guide for the Care and use of Laboratory Animals (Eighth Edition 2011) and were approved by the Animal Care and use Committee of the I.Beritashvili Center of Experimental Biomedicine.

**Statistical Treatment.** Results were treated statistically by means of Student’s t test.  $*=p<0.05$  and  $**=p<0.01$  were taken as the levels of significance.

**Results and discussion.** It should be noted that numerous studies attempted to better understand the mechanisms and pathophysiology of human depressive state. However, the problem is still far from the final decision. Undoubtedly, development of animal models reproducing most symptoms of human depression is topical and there were many attempts in this direction [3,4,6,7,9,10,14-18]. Despite this the potential need for the animal model of depression more appropriately reproducing all the signs of human depressive state still remains. Our study is the one more attempt to this direction. Like previous authors in the present study we used genetically determined natural differences in the behavior of rats in the FST [19]. Therefore, “non-depressive” and “de-



**Fig. 1.** Percent relationship between activity (assessed by swimming and climbing) and immobility times in FST in “non-depressive” (A) and “depressive” (B) rats

1. Data for the first day experiment in FST, 2. Data for the second day experiment in FST. \*\* = $p < 0.01$ .

pressive” rats were selected by means of FST device. This test gave us possibility to select rats as “depressive” and “non-depressive” according to the level of immobility, helplessness in the inescapable situation and the speed of surrender.

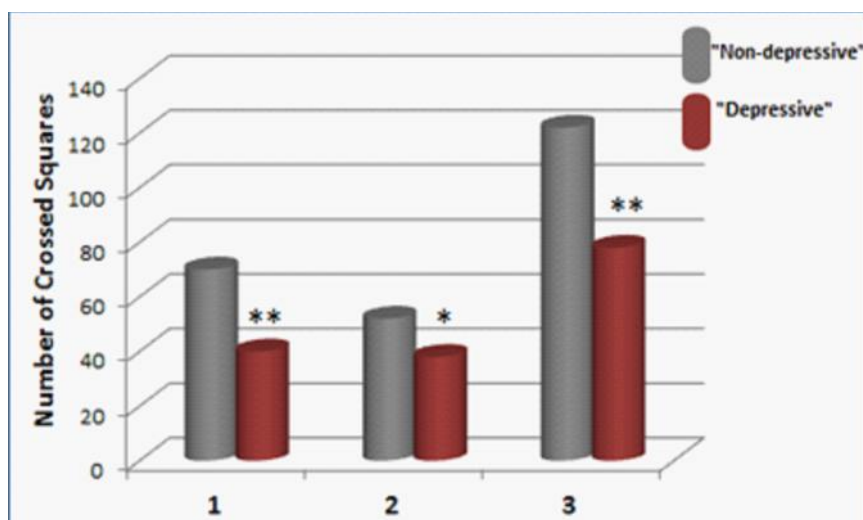
It appears that the main time in the FST apparatus is spent by one part of rats in swimming and searching for a way out of the cylinder. Rats swim and climb mainly during the first and second experimental trials and these behaviors take 64% and 67% for the first and second day of experiments respectively (Fig.1A, gray columns). Duration of their immobilization time is very short - 36% of 15 min for the first day and 33% of 5 min for the second day experiments (Fig.1A, red columns). Therefore, these rats constituted the low immobility “non-depressive” control group.

Another part of the rats are naturally prone to depressive state, which manifests itself in the fact that after inserting into the FST cylinder they surrender very soon and spend the most of their time in the water in hanging state. Immobilization time for such rats is very high – 70% of 15 min for the first day, 73% of 5 min for the second day (Fig.1B, red columns). Time spent in swimming and climbing is very short - 30% of 15 min for the first day, 27% of 5 min for the second day (Fig.1B, grey columns), therefore these rats constituted high immobilization “depressive”

group.

FST is a common tool widely used now for evaluation of the efficacy of anti-depressant drugs and the effects of various behavioral and neurobiological manipulations in basic and preclinical research [12, 20-22]. It is described as a rendering situation, in which “behavioral despair” is induced; that is, the animal loses hope to escape the stressful environment [11, 12]. Because of popularity of FST there is a wealth of data regarding the effects of various anti-depressants in the FST. This allows researchers to compare and contrast their own results with the others (see 19 for review). Another area where the FST is used is neurogenetic research, in which the genetic basis of depression-related behaviors is investigated. In this regard, the FST has proven to be useful in basic research related to the neurobiology and genetics of mood disorders [22].

It is a well known fact that depressive patients are characterized by some behavioral disorders, reduced general activity and locomotion and general loss of interest among them. These behavioral items are readily to investigate in rodents by OF test designated for the measuring of behavioral responses such as locomotor activity, exploratory and emotional behaviors. OF is also used as a measure of anxiety and fear. This technical device is based on well-



**Fig. 2.** Changes in horizontal locomotor activity according to the number of crossed squares in high immobilization “depressive” rats.

1. Data obtained for the first 5 min of observation, 2. Data obtained for the second 5 min of observation, 3. Data obtained for the whole 10 min of observation. \* =  $p < 0.05$ .

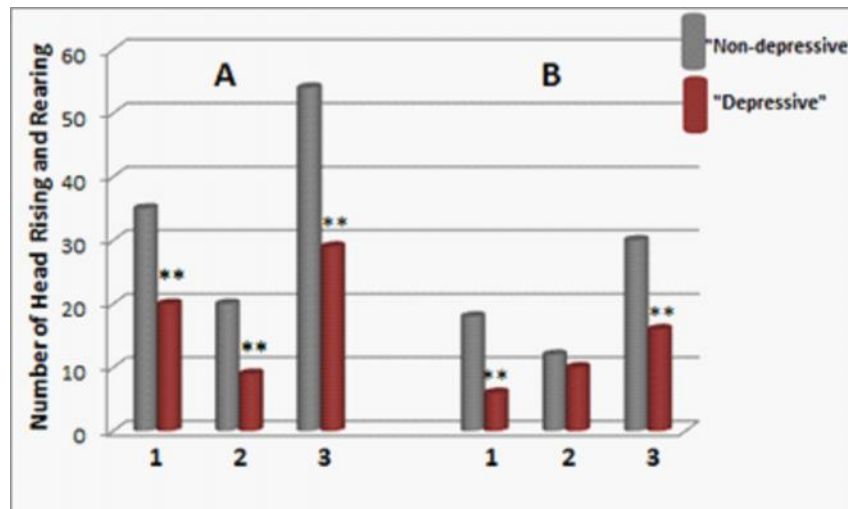
known fact that rats naturally have a tendency to avoid brightly illuminated, novel, open spaces. It is a stressful situation that naturally does produce inhibition of exploratory behavior at first, but in healthy rats OF arena after short period of staying in it becomes not extremely stressful. In other words, competition between exploratory and fear emotions appear at first and finally rats start exploration of novel space, that is healthy animals are aware in real perception of the level of stressfulness of OF. Therefore, it was very interesting for us to investigate: character of behavioral disturbances of “depressive” rats in OF area; degree of perception of novelty and/or stressfulness of OF situation and possibility of “depressive” animals really percept the level of stressfulness in the novel surrounding. These questions were posed by us early too in animal model of depression characterized by deficiency of brain monoamine/serotonin content [6].

It appeared that rats from the low immobilization and high immobilization groups significantly differ each from other in their behavior in OF test. Namely, Fig.2 represents the data concerning the changes in horizontal locomotor and exploratory activity according to the number of crossed squares in these two groups of animals. For the parallel assessment of the

process of habituation to the OF area that can be a direct indicator for the degree of perception of novelty and/or stressfulness of OF situation the data were analyzed for the first 5 min, second 5 min and whole 10 min of observation. Data presented in Fig.2 clearly show that high immobilization “depressive” rats are less active in the OF area. It is manifested in significant reduction of the number of crossed squares in comparison to the control “non-depressive” rats.

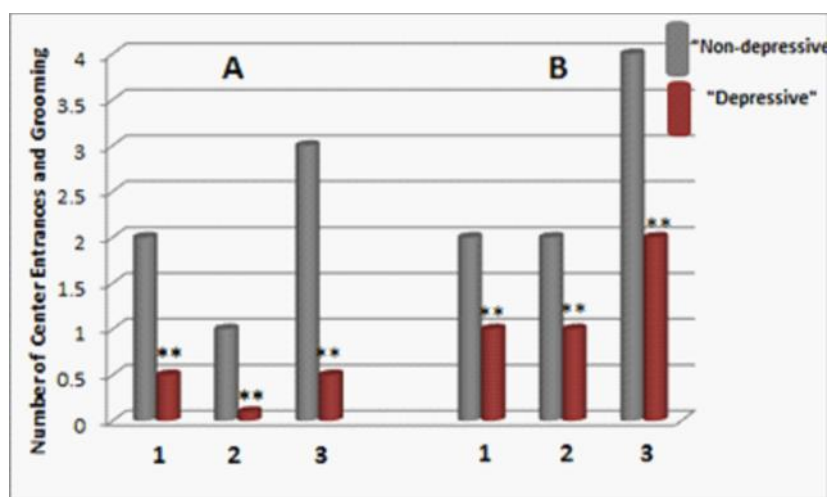
Differences between the data of control and experimental groups are approximately equal for all periods of exploration (Fig.2.1; Fig.2.2 and Fig.2.3). The number of crossed squares in high immobilization “depressive” rats is by 25% less for the first 5 min periods of exploration, 20% less for the second 5 min period of exploration and consequently by 22.5% less for the whole 10 min period of exploration in comparison to the low immobilization “non-depressive” rats (Fig.2). The process of habituation to the OF area, assessed by the number of crossed squares, was significantly faster in control “non-depressive” rats, than in high immobilization “depressive” rats.

The qualitative values of the indices for vertical activity in OF area (head rising, rearing) were also sharply reduced in “depressive” rats. Fig. 3 repre-



**Fig. 3.** Changes in vertical locomotor activity according to the number of head risings and rearing in high immobilization “depressive” rats.

1. Data obtained for the first 5 min of observation, 2. Data obtained for the second 5 min of observation, 3. Data obtained for the whole 10 min of observation. \*\* =  $p < 0.01$ .



**Fig. 4.** Changes in motivational emotional behavior assessed by the rate of the entrances in open field center and grooming in high immobilization “depressive” rats

1. Data obtained for the first 5 min of observation, 2. Data obtained for the second 5 min of observation, 3. Data obtained for the whole 10 min of observation. \*\* =  $p < 0.01$ .

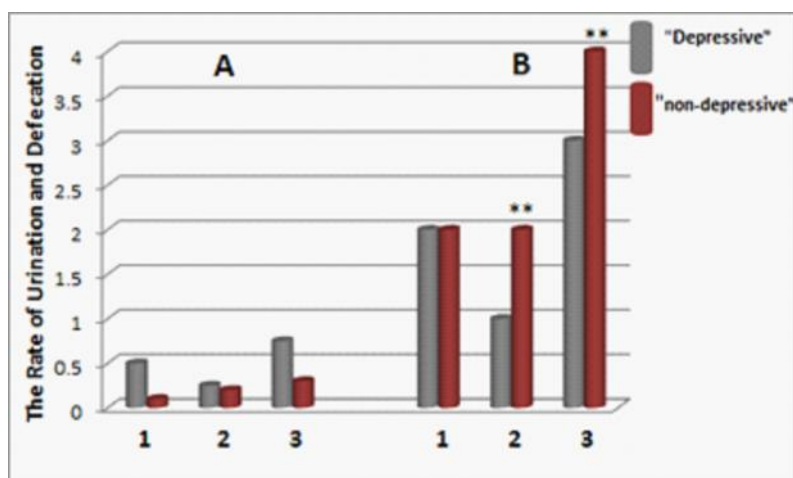
sents the changes in the number of head rising and rearing (vertical standing) and results of statistical treatments show that the number of head risings undergoes to the gradual significant reduction during the first and second 5 min periods of exploration.

The value of this index becomes two times reduced in high immobilization “depressive” rats in comparison to low immobilization “non-depressive” ones (Fig.3.A.1 and 3.A.2). Thus, the process of habituation to the OF area assessed by the number

of head risings is also faster in control “non-depressive” rats. As for the number of rearing (vertical standing) this index becomes 2 times reduced in “depressive” rats (Fig.3B).

In conclusion, presented results indicate that horizontal and vertical locomotor activity is reduced and exploratory behavior becomes suppressed in the high immobilization depressive rats.

The parameters of OF test indicating the changes of motivational emotional behavior of animals were



**Fig. 5.** Changes in motivational emotional behavior assessed by the rate of the urination and defecation in high immobilization “depressive” rats.  
1. Data obtained for the first 5 min of observation, 2. Data obtained for the second 5 min of observation, 3. Data obtained for the whole 10 min of observation. \*\* =  $p < 0.01$ .

analyzed separately. Those are the number of entrances in the center of OF area, grooming behavior, defecation and urination. It appears that the number of entrances in the center of open field area undergoes to the significant changes, namely its value for all three periods of observation equals almost to zero (Fig.4A).

In other words, the intensity of fear emotion becomes so elevated in „depressive” rats that they do not enter the center of OF during the whole period of observation, and if so they are moving mainly at the periphery of OF area in contrast to the control „non-depressive” rats.

It is worth mentioning that, naturally, the rate of grooming in OF area is not high in intact rats. The main reason for this is the specificity of OF arena because it represents the new highly illuminated stressful situation for rats and for this reason the majority of control animals are showing grooming behavior only 3-4 times during the whole period of observation. This value becomes lesser in the high immobilization “depressive” rats. Reduction is evident in the first and second 5 min periods and consequently for the whole 10 min of observation (Fig.4B). It appeared that the rate of urination was also very low in control non-depressive rats and became lesser in the high immobilization “depressive” rats in the first and second 5 min periods of exploration and consequently for the whole 10 min of

observation in OF area (Fig.4A). The rate of defecation was increased in the high immobilization “depressive” rats in comparison to the data of control non-depressive rats (Fig.5B).

Thus, we found that high immobilization, depressive rats” exhibit significant diminution of locomotor activity. They are apparently depressed in OF during the whole period of staying in it. These findings are in agreement with the results obtained earlier on Flinders’ sensitive line rats [2]. This exaggerated immobility is not unexpected; there is literature indicating that cholinergic agonists accentuate and cholinergic antagonists reduce swim test immobility. Hasey and Hanin [2, 23] confirmed the acute depressive effects of cholinergic agonists (i.e., physostigmine) on swim test immobility. These investigators also reported that these cholinergic effects could be partially counteracted by noradrenergic manipulations, and they proposed a balance model reminiscent of the original adrenergic/cholinergic balance model of affective disorders [2].

It was also shown in the present work that high immobility “depressive” rats are characterized by significant elevation in the level of fear emotion that is expressed in the sharp decrease of the number of center entrances, grooming and increases in the number of defecation. Changes of exploratory and emotional

behavior were studied for the first time by us. These are very significant measures for depressive-like state in animals and we found very interesting results in these respect. Namely, it was shown that “depressive” animals exhibit significant decrease of grooming behavior an indicator in norm of animal’s desire put himself in more comfortable state. Apparent reduction were evident in the frequencies of center entrance and the time of staying in the center of OF, in other words, high immobility “depressive” rats exhibited complete loss of exploratory motivation. We think that the loss of interest is not related to the enhancement of fear emotion only. Such a notion is directly supported by unchangeable values of the rate of urination. These findings indicate that high immobility “depressive” rats are characterized by behavioral ‘despair’ or “refractory loss of interest”.

In conclusion, genetically determined natural differences in rats behavior in the FST, manifested in the level of immobility, helplessness in the inescapable situation and the speed of surrender to this situation, are those significant factors that makes possible to reveal more substantial differences in the OF behavior between “non-depressive” and “depressive” rats. Namely, it was shown by us that rats with high immobilization, which surrender more quickly in the FST, manifest depressive-like behavioral disturbances in the OF test. They are characterized by significant suppression of general activity, locomotor and exploratory behavior and elevation of fear emotion. The ability for real assessment of the level of danger in the new, extremely illuminated area is much diminished and they actually underestimate the surrounding situation.

*ადამიანისა და ცხოველთა ფიზიოლოგია*

## კვლევითი, ლოკომოტორული და მოტივაციური-ემოციური ქცევის ცვლილებები მაღალი იმობილიზაციის “დეპრესიულ” ვირთაგვებში

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(წარმოდგენილია აკადემიის წევრის თ. ნანიშვილის მიერ)

წინამდებარე შრომაში შეისწავლეს შავგულიძე და კვლევითი დარღვევების ხასიათი მაღალი იმობილიზაციის „დეპრესიულ“ ვირთაგვებში, კერძოდ, კვლევითი, ლოკომოტორული და მოტივაციური-ემოციური ქცევის ცვლილებები ღია ველში. მაღალი იმობილიზაციის „დეპრესიული“ და დაბალი იმობილიზაციის „არადეპრესიული“ ვირთაგვების გადარჩევისთვის პორსოლტის იძულებითი ცურვის ტესტი გამოიყენებოდა. ნაჩვენებია იქნა, რომ „დეპრესიული“ ცხოველები ლოკომოტორული ქცევის

რედუქციით ხასიათდებიან. ისინი აჩვენებენ აგრეთვე გრუმინგების მნიშვნელოვან დათრგუნვას, რაც მიუთითებს, რომ „დეპრესიულ“ ვირთაგვებს დაქვეითებული აქვთ საკუთარი თავის მოვლის და უფრო კომფორტულ მდგომარეობაში ყოფნის სურვილი. მკვეთრად არის შემცირებული ღია ველის ცენტრში შესვლის სიხშირე. სხვა სიტყვებით რომ ითქვას მაღალი იმობილიზაციის „დეპრესიული“ ვირთაგვები აჩვენებენ ინტერესის სრულ დაკარგვას და კვლევითი მოტივაციის რედუქციას. მიღებული შედეგები მიუთითებს, რომ მაღალი იმობილიზაციის „დეპრესიული“ ვირთაგვები ქცევითი „უძლურებით“ და „ინტერესის რეფრაქტორული დაკარგვით“ ხასიათდებიან.

ამრიგად, გენეტიკურად განპირობებული ბუნებრივი განსხვავება ვირთაგვების ქცევაში, იძულებითი ცურვის ტესტში, რაც ვლინდება იმობილიზაციის დონეში, თავდაუღწევად წყლით სავსე გარემოში განვითარებულ უძლურებაში და ამ სიტუაციისადმი დანებების სინქარეში, აღმოჩნდა ის მნიშვნელოვანი ფაქტორი, რომელიც შესაძლოა ხდის უფრო საფუძვლიანი ქცევითი განსხვავებების გამოვლენას მაღალი იმობილიზაციის „დეპრესიულ“ და დაბალი იმობილიზაციის „არადეპრესიულ“ ვირთაგვებს შორის. კერძოდ, შრომაში ნაჩვენებია იქნა, რომ მაღალი იმობილიზაციის ვირთაგვები, რომლებიც უფრო ადვილად ნებდებიან იძულებითი ცურვის ტესტში, ავლენენ დეპრესიული დაავადების მსგავს ქცევით დარღვევებს ღია ველში. „დეპრესიული“ ვირთაგვებში დათრგუნულია ახალი, ზედმეტად განათებული, არედან მომავალი საფრთხის აღქმის უნარი, ისინი რეალურად ვერ აფასებენ გარემომცველ არეს.

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