Relationship between intestinal microbiota and memory and anxiety: effects of antibiotics and probiotics

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Mashhad city

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بسم الله الرحمن الرحيم

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Disclaimer : Our main version of this paper is still in Persian. This is a messy version just for a little introduction. It was translated by Google Translate. We will translate it ourselves in the near future.

Dedicated to our dear and kind parents

Abstract

The aim of this study was to discover the relationship between intestinal microbiota and long - term spatial memory and anxiety . In a trial of20 Balb / c mice :into four groups1 Control .2 Antibiotics .3 Low dose probiotics .4 .

High - dose probiotics were administered. First, all groups except the control group underwent oral gavage with a mixture of antibiotics. At the end of the antibiotic course, they were tested for behavioral , memory, and anxiety tests; The probiotic groups then received a probiotic mixture at a given dose. At the end of the probiotic course, memory and anxiety of these groups were also assessed. Finally, the results of the tests showed a significant increase in anxiety in the probiotic groups, and no .significant difference was observed in the spatial memory of the four groups

English keywords

g ut microbiota, HPA axis, s patial memory, anxiety, vagus nerve, antibiotic, probiotic, Morris Water Maze (MWM), Elevated Plus Maze

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Introduction

,A microbiota is a collection of microorganisms, including bacteria, fungi, archaea and viruses. These microorganisms live in various organs of the body that are in contact with the external environment, including the surfaces and deep layers of the skin, mouth, lungs and especially in the human intestine. There are tens of trillions of them . This microbial population, a significant part of which is transmitted from generation to generation, plays a key role in maintaining human health. Their imbalance is associated with various diseases such as diabetes, obesity , allergies ,and cancers . Studies also show that human coexisting bacteria affect **various social emotional, neurological and anxiety behaviors in** humans by affecting the development and function of the human brain . Various human microbiome projects that are underway show that microbiota are involved in **the evolution of the immune system, the fight against pathogenic microbes, the body's basal ,metabolism, the digestion of unusable substrates, the production of vitamins the regulation of hormone secretion,** and the general maintenance of Human survival plays an important role . Considering that the microbiome of every human being in each region and country is different and unique and has a direct impact on the process of diseases and their type of treatment, studying the microbiome of people in the country and investing in this field is very important . (Source: Research Institute Immunology and Infectious Diseases) Recent research has shown an association between the gut microbiota of animals and their central nervous system)CNS which may be related to the ,(HPA .(hypothalamic-pituitary-adrenal) axis One method of this connection is the secretion of active neuronal compounds such

as metabolites and neurotransmitters by bacteria. Therefore, it is important to . identify the factors that upset the balance of the gut microbiota and prevent them In this study, an attempt has been made to understand the possible effects of these changes on spatial memory and anxiety by making changes in the bacterial ,population of intestinal microbiota. To make changes to the gut microbiota of mice a course of a mixture of four antibiotics was first considered. After passing this course, a new course of consumption of a mixture of two probiotics took place. In the final days of each consumption period, the Morris and Maze Plus maze tests were taken from the mice to assess spatial memory and anxiety, respectively. These tests are one of the most famous behavioral tests in the field of cognitive neuroscience and have been used in valid scientific research to study the cognitive factors in rodents. Due to the subtlety and specialization of microbiology, accurate study of the composition of the gut microbiota is not easily possible and requires .advanced equipment and technologies that are only available in certain countries Therefore, one of the important challenges in such studies is the proper and accurate evaluation of the composition of the intestinal microbiota. In the field of neuroscience, the preparation and maintenance of behavioral tests such as the Morris water maze is relatively costly and difficult. For this reason, researchers are advised to carefully estimate the facilities and equipment they need before starting .their research so as not to encounter any problems in the middle of the research Finally, we would like to thank Mr. Mohammad Vahidi Arbabi and Mr. Erfan .Najafzadeh who helped us a lot to make this research fruitful

Statement of the problem

.One of the most well-known factors that upset the microbiota balance is antibiotics Contrary to popular belief, there is controversial research showing that antibiotics] . do not affect spatial memory by disrupting the gut microbiota16 However, there [is disagreement in the articles regarding anxiety. However, an unbalanced intestinal microbiota needs treatment, and according to research, one of the ways to prevent and treat it is to use probiotics. However , there is disagreement about the relationship between spatial memory and anxiety with the use of probiotics and subsequent microbiota changes. Given the high importance of intestinal microbiota on human health, as well as the increasing and increasing use of antibiotics despite : their destructive effects on the intestinal microbiota, the question arises

Does taking probiotics eliminate the possible negative behavioral and cognitive ?effects of antibiotics on the gut microbiota

background research

Fossil research and fossil research show that the first bacteria appeared on Earth .about four billion years ago; The Earth's atmosphere was not formed at that time Millions of years later , cyanobacteria emerged, forming the ozone layer and the Earth's atmosphere, which marked the beginning of the evolution of oxygen- .dependent organisms

There is a theory that when bacteria began to evolve, they formed modern cells, or organ cells. The fact that mitochondria are genetically very similar in size and ,genetically to certain species of bacteria is a confirmation of this theory. In fact bacteria are the ancestors of our cells. During the evolution of bacteria and the creation of the Earth's atmosphere and the formation of living organisms, the bodies

of extracellular organisms gradually lived with bacteria; And the trillions of bacteria .and germs that live with us in our bodies are examples of this

The bodies of most living things are surrounded by a large number of microbes. Our knowledge of the relationship between living organisms and the microorganisms present in their bodies has increased dramatically in recent years. Scientists were first able to discover the relationship between the microbiome (microbes that live in the mouth, intestines, genitals and skin) and the digestion of food and digestion in living organisms. Then they found out the relationship between the microbiome and the immune system of living organisms. Today, scientists are using microbiomes to .try to boost the immune system in living things

In fact, we humans are " superorganisms" " that are run bymicroorganisms that " live inside our bodies! As mentioned, many discoveries have been made about the microbiome. Scientists today have discovered that the microbiome is involved with .our nervous system, and more research is being done on this subject

Microbiome growth appears to begin at birth, although a number of studies have ,challenged this by identifying microbes in tissues such as the placenta. Interestingly the type of delivery also affects the composition of the microbiome, and the intestines of infants born by natural childbirth are colonized later than those of infants born by cesarean section due to failure to pass through the birth canal. When a baby is born, a lot of germs enter the body through breast milk, so one of the disadvantages of using powdered milk is that the germs are not transmitted from mother to child. In general, after birth, breast milk and the environment are the two .main factors affecting the composition of microbiota in infants

In early infancy, the composition of microbiota is less diverse and often includes

proteobacteria andactinobacteria During the first year of life, microbial diversity . increases and the microbiota composition tends towards a specific composition; But following the onset of disease, the use of antibiotics, and changes in diet, the .composition of the microbiota of the gastrointestinal tract changes

The microbiome of each organism is different from other organisms for several reasons; There are also various factors that affect the composition of the intestinal :microbiome. These factors include

Diet : For example, the intestinal microbiome of carnivores is different from that of vegetarians such as cattle and dogs, and one of the main reasons is the difference in their diet. This factor is also effective for humans; For example, the intestinal microbiome of a Japanese person is completely different from the intestinal .microbiome of an Iranian person due to its special diet

) **Host genotype** : The human genusDNA produces actions and reactions on the (. microbiome by producing specific proteins

Physiology : The microbiome is associated with various tissues and organs in the body. For example, the human gut microbiota interacts with and affects theHPA axis.

Immune system : There is a combination of beneficial and harmful bacteria in the ;human intestine; If the composition of intestinal bacteria has a healthy balance Prevents infection and accumulation of harmful bacteria and thus strengthens the immune system. In poultry farms today, when the chicks hatch; Probiotics are sprayed on them. Because chickens have a weak immune system at birth, the microbial composition of their intestines does not have the necessary balance to

.counteract the growth of harmful kettles

Environment : Animals that live on land have different microbiomes than animals that live in water due to different bacterial growth conditions, temperature, and .many other factors

Pathology : for example, those with gastric ulcer; Bacteria that live in their gut enter the bloodstream, and because some gut bacteria are pathogenic; They cause various diseases. It is also possible for a gastrointestinal, immune or neurological disease to ,affect the gastrointestinal tract; Cause changes in the microbiome population. Also the microbiome is the source of some diseases. Gangaria disease is one of these diseases. A person with gangrene opens the door to deeper spots for sporophytic bacteria that live on the skin due to deep wounds on the body. The entry of these bacteria into deeper areas causes infection, and eventually the infected organ dries .out and falls off

Of course, there is a two-way relationship between these factors and the microbiome Figure)1 .(

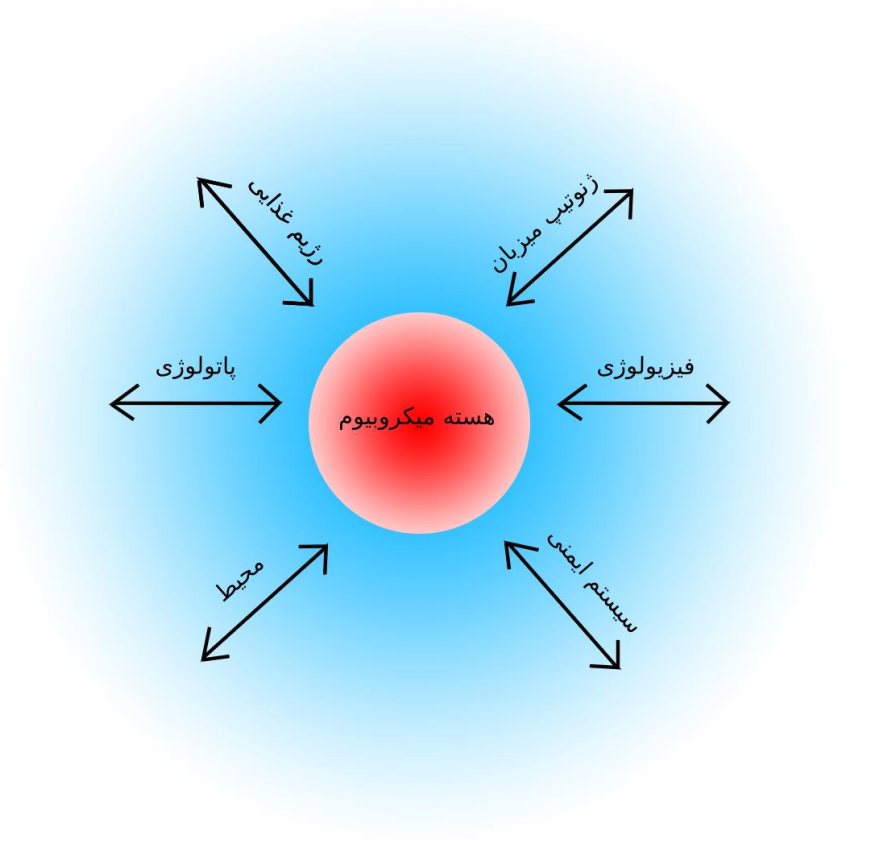


Figure1 Factors affecting the intestinal microbiota :

Relationship between intestinal microbiota and brain

According to recent research, the billions of microbes that live in the gut can play a key role in supporting the formation of new nerve cells in the adult brain. It is also possible to prevent memory loss in old age and to repair and regenerate cells. Nerve help after injury30 [ ] .

Intestinal microbiota is also influential in cognitive diseases such as autism, and scientists in recent years have concluded that autism is one of the diseases associated

with altered intestinal microbiota. In fact, scientists have discovered that a decrease in the population ofAkkermansia muciniphila andBifidobacterium is seen in the .feces of children with autism

Other examples of the relationship of microbiota to the nervous system include the effect of metabolites on neurons; These metabolites are small, light chains of fatty .acids produced by bacteria that activate neurons

Association of microbiome withHPA axis

HPA stands forHypothalamus, Pituitary, and Adrenal. These three parts affect each other by secreting hormones under different conditions, such as stressful conditions . But the components of this axis are not only affected by each other, but have been proven to be related to other parts; One of these parts that may be related to theHPA axis " is a group of microbes that are in our gut and are calledGut microbiota Intestinal microbiota can possibly control the secretion of hormones ." and affect the activity of immune cells through the hypothalamic-pituitary-adrenal axis during times of stress. Activity-driven corticosteroid hormones releasedHPA They can also regulate the gut microbiota. According to research, colonization of Bifidobacterium infantis in the gut improves behavioral and stress- related brain defects . Some effects of microbiota in the brain through the axisHPA Which can change the behavior of the host. Figure2 shows the chemical relationship of the nervous system with the intestinal microbiota. As shown in the figure; The hypothalamus secretes a chemical calledCRF which stimulates the pituitary gland , to secrete ACTH. ACTH affects the adrenal gland and the adrenal gland begins to

. secrete cortisol, which affects the gastrointestinal tract and intestinal microbiome

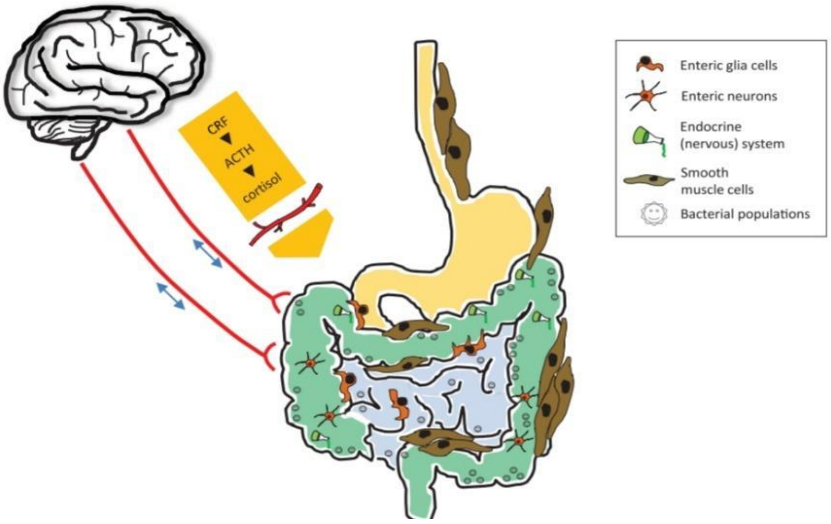
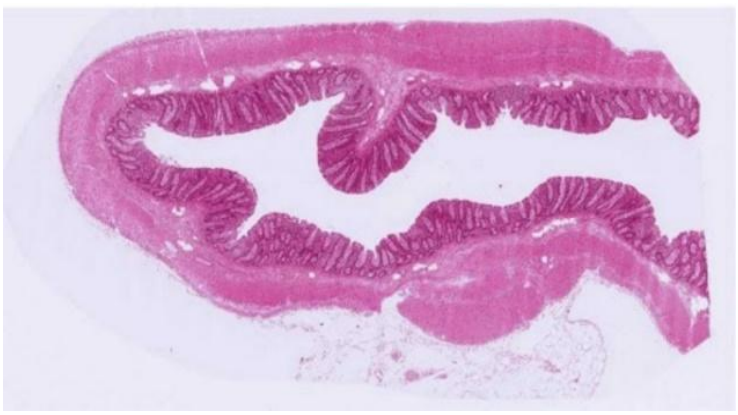
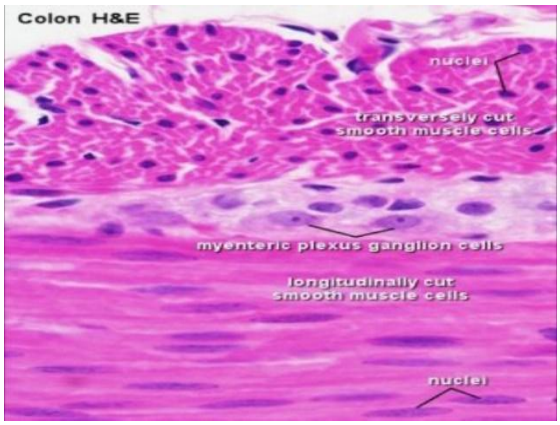


Figure2 Relation of microbiome to :HPA axis

How the nervous system communicates with the microbiome

InFigures 3.1 and3.2 you can see a section of colon tissue. The colon, like other , ,parts of the gastrointestinal tract, is made up of connective, muscular, submucosal .and mucosal tissues from the outside to the inside





Figures 3.1 and3.2 respectively ,

Nerve tissue in the submucosal tissue secretes substances into the gastrointestinal

tract. Microbes are associated with this nerve tissue. One of these communications .is through the secretion of chemicals

,In addition, the gut microbiota is associated with the hippocampus, amygdala hypothalamus, and frontal cortex of the brain, but how this is communicated has not .yet been determined

Also, a group of intestinal microbes can produce intestinal hormones or peptides] .such as arxin, galanin, ghrelin, gastrin and leptin11 [

The intestinal microbiota affects the central nervous system through the intestinal nervous system. Due to the similarity of the intestinal nervous system to the central nervous system in terms of autonomy, the intestinal nervous system is known as the second brain. Figure4 deals more fully with the relationship between the gut .microbiota and the nervous system

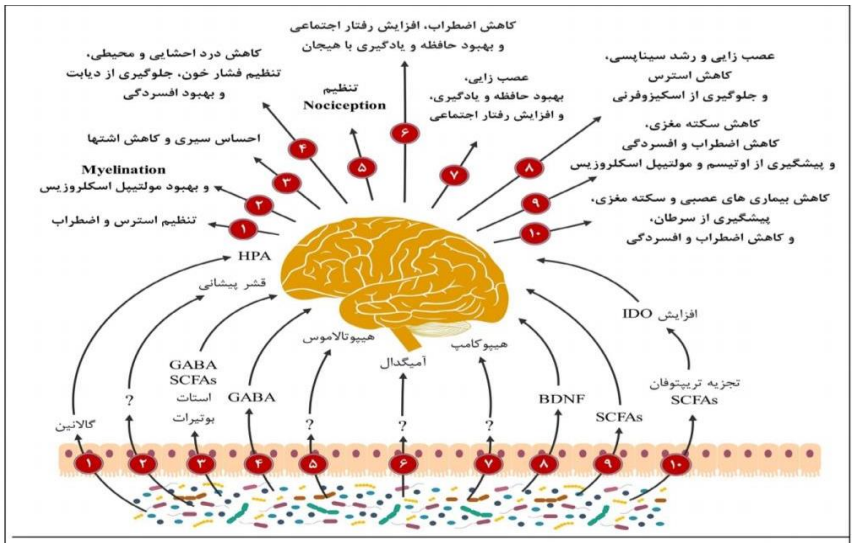


Figure4

Targets

Overall Objective : To make changes in the bacterial population in the intestinal microbiota of mice and finally to investigate the effect of these changes on spatial) memory and anxiety. Using Morris water maze and suspended maze plusElevated plus tests (

:Minor Objectives

1 Evaluation of the effect of antibiotic mixture on reducing the population of .bacteria inhabiting the myelin gland, on spatial memory and anxiety

2 Reduce the population of natural bacteria living in the intestine of Mays with a mixture of antibiotics, then replace that population with neurotransmitter-secreting bacteria in the probiotic mixture, and finally investigate the effects .of these actions on spatial memory and anxiety

theories

- Taking a course of a mixture of four antibiotics can increase anxiety in mice .by upsetting the balance of the gut microbiota
- Taking a course of a mixture of two probiotics after a course of antibiotics regenerates the gut microbiota and causes the gut to colonize with neurotransmitter-secreting bacteria. The neurotransmitter-secreting bacteria

then help to strengthen long-term spatial memory and reduce anxiety in mice .by communicating with the brain

method

Under the supervision of the head of the laboratory, twenty Maybus C were first , divided into four groups of control, antibiotics and two groups of probiotics. Then for11 days, a mixture of four antibiotics, amoxicillin, vancomycin, gentamicin and ciprofloxacin, was orally gavaged to the mice of the antibiotic group and the two probiotic groups; The control group was also gavaged with normal saline solution for the same period. From the twelfth day onwards, the Morris water maze and the Elevated plus maze tests were taken from the control and antibiotic groups to assess spatial memory and anxiety . From the twelfth dayonwards the ,probiotic group was not given a mixture of antibiotics and the probiotic course for them started from this day. Highdose and low dose probiotic group were gavaged. Finally, in the last days of the experiment, the behavioral tests of Morris and Maz Plus were taken from the probiotic groups. It should be noted that the two Mays contracted a non-communicable disease and lost their lives due to the stress of moving to a new .environment and being isolated

antibiotics

.Some intestinal bacteria affect the nervous system by secreting neurotransmitters) Table1 shows a general form of neurotransmitters and the bacteria involved in (.their secretion

Neurotransmitter	Bacteria Strain
Dopamine	Bacillus cereus Bacillus mycoides Bacillus subtilis Escherichia coli
Acetylcholine	Lactobacillus plantarum
Histamine	Lactobacillus plantarum Lactobacillus hilgardii Lactobacillus mail Lactococcus lactis subsp.cremoris Lactococcus lactis subsp.lactis Streptococcus thermophilus
Serotonin	Lactobacillus plantarum Lactococcus lactis subsp.cremoris Escherichia coli Streptococcus thermophilus

Norepinephrine	Escherichia coli Bacillus subtilis Bacillus mycoides
Neurotransmitter	**Bacteria Strain**
GABA	Lactobacillus delbrueckii subsp Lactobacillus rhamnosus Lactobacillus reuteri Lactobacillus plantarum Lactobacillus paracasei FRI Lactobacillus buchneri Lactobacillus brevis Bifidobacterium adolescentis Bifidobacterium angulatum Bifidobacterium dentium Bifidobacterium infantis Bulgaricus Streptococcus thermophilus

Table1] Bacteria and neurotransmitters secreted by them :1] [12 [

Each of these transporters perform functions in the human body and affect different parts of the central and peripheral nervous system. But among these ,neurotransmitters are norepinephrinedopamine ,and serotonin. There are three neurotransmitters that play the most important role in the cognitive part of the .central nervous system

Norepinephrine : It plays a role in factors such as anxiety, concentration, alertness

.and sleep

Dopamine : It plays a role in factors such as anxiety, social relationships, self-esteem, concentration and diseases such as .Parkinson's

Serotonin : It plays a role in factors such as memory, learning, depression, emotions .and sleep

According to research, the gut microbiota changes under the influence of various factors, including antibiotics, its amount and variety; Antibiotics are an important class of drugs that fight bacterial infections in the body and kill bacteria or prevent them from growing and multiplying. Due to the fact that the use of antibiotics has a faster effect on the intestinal microbiota than other methods, in this experiment, a .mixture of antibiotics was used

Because each antibiotic works on a specific range of bacteria, and one antibiotic may be useless against another bacterial infection, each group of these bacteria is resistant to several antibiotics and to a number of others. They are more sensitive than antibiotics. Therefore, considering the bacteria that are more involved in the ,secretion of three neurotransmitters norepinephrine, dopamine and serotonin ,antibiotics were selected to reduce the population of these bacteria. These bacteria) along with the antibiotics prescribed for them, are listed in Table2 .(

Bacteria genus	Antibiotics
Escherichia	)Ciprofloxacin
Streptococcus	Vancomycin

Bacillus	Gentamicin
Lactobacillus and Bifidobacterium	Amoxicillin

Table2] Bacterial genera and antibiotics selected for each :2] [3] [4] [5] [6 [ ]7] [13] [14 [

The best way for antibiotics to work on the body's microbial flora is based on a mixture of water. Therefore, in order to harm this population of bacteria, the mentioned antibiotics should be orally gavaged to the mice at different times, in ,different doses and orally (mixed in water). Considering the average weight of mice) a specific dose was considered for each antibiotic, which is specified in Table3 . (

Antibiotics	Dosage	Amount in mixture
Amoxicillin	1.5 mg / ml	0.15 mg
Gentamicin	0.0125 mg / ml	0.025 mg
Vancomycin	1.5 mg / ml	0.15 mg
Ciprofloxacin	1.5 mg / ml	0.15 mg

Table3] Specified doses for antibiotics :8 [

Probiotics

After using the antibiotic mixture, the population of the desired bacteria was reduced in all three groups; A replacement for the lost bacterial population of microbiota must be identified immediately; If this is not done quickly, pathogenic bacteria may replace them. Therefore, in this experiment, probiotic drugs containing bacteria that secrete neurotransmitters such as GABA and serotonin were used because they are likely to affect cognitive activity. The two probiotic drugs used areComflor and BioFem .

Table4 lists all the species in the two probiotic drugs used . But only species

L. reuteri, L. rhamnosus, L.plantarum , Streptococcus thermophilus, B.infantis

They secrete neurotransmitters, and the other species do not secrete neurotransmitters, simply because no probiotic drug has been found that contains .only the species in question; These drugs were selected

Probiotic	Bacteria
Comflor	Lactobacillus plantarum
	Lactobacillus casei
	Lactobacillus acidophilus
	Lactobacillus bulgaricus
	Bifidobacterium longum
	Bifidobacterium breve
	Bifidobacterium Infantis
	Streptococcus thermophilus
Bio Fem	Lactobacillus Rhamnosus
	Lactobacillus Reuteri

Table4

It should be noted that probiotics are given to two of the four groups of mice. These two groups were given two different doses of probiotics to increase the population of neurotransmitter-secreting bacteria in different proportions for each group; One group was given a low dose of probiotics and the other group was given a high dose of probiotics. After the probiotic course, anxiety and spatial memory tests were taken from these two groups . Probiotic doses are shown in Table**5** .

Group	Probiotic	Dosage
High dose	Biofem	7.5×10^5 CFU
	Comflor	$3{,}375 \times 10^8$ CFU
Low dose	Biofem	2.5×10^5 CFU
	Comflor	1.125×10^8 CFU

Table5 Specified doses for probiotics, according to the prescription of each drug :

Finally, you can get an overview of the groups studied in Table6 you have. Each group contains4 . mice

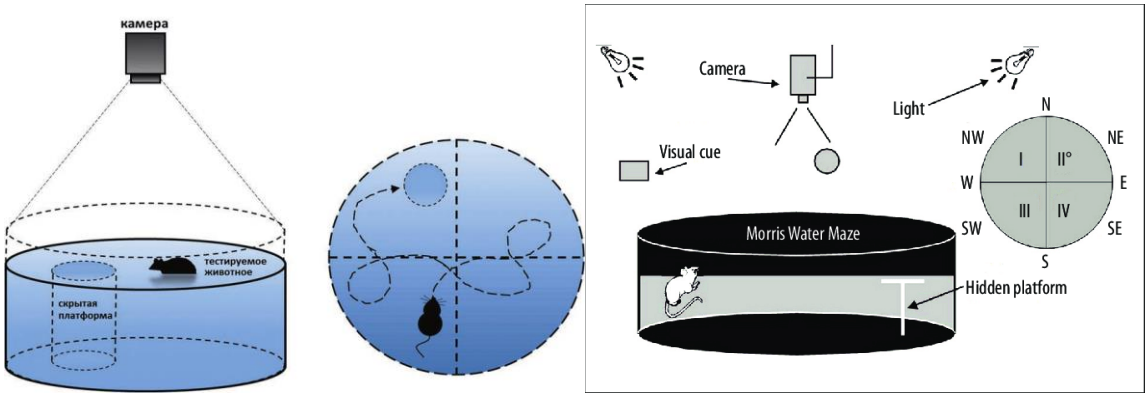
Probiotic dose	probiotic	Antibiotics	group
-	✗	✗	control group
-	✗	✓	Antibiotic group
Low dose	✓	✓	Le Dos probiotic group
High dose	✓	✓	Dosage probiotics group

Table6 Experimental groups :

Cognitive factors and memory and anxiety tests

The selected cognitive factors are long-term spatial memory and anxiety, and the selected tests are Maurice Blue Maze tests andElevated Plus maze At the end of the antibiotic and probiotic .

courses, all the mice, Morris water maze and Elvid Plus tests were taken. Regarding the Morris ,test, it should be noted that this test is one of the most familiar tests in the field of neuroscience which is performed to assess spatial memory in rodents. In addition, this test shows the effects of improving the memory or forgetfulness of drugs, as well as the effects of genetic manipulation related to hippocampal function. Also unlike mazes like the eight arms andY , .this test does not require a hunger test for mice



Figures 5.1 and5.2 respectively : Morris Blue Maze ,

Elevated Plus test is also the most important application in examining the effect of drugs on .anxiety. Therefore, this test has been used to evaluate the anxiety factor in mice

Test schedule and schedule

Summary

1 Preparation of mice and equipment

2 Getting the mice accustomed to the environment and the experimenter)Adaptation (

3 Antibiotic course

4 Take tests from the antibiotic and control groups

5 Probiotic course

6 Take a test of probiotic group mice

7) Data analysisOne-way ANOVA statistical test, fixed factor (

Procurement of equipment and preparation of mice

Equipment

Necessary equipment includes cages and their accessories, gavage accessories such as special needles, as well as Morris water maze tests andElevated plus maze .

Antibiotic course

Mixtures of antibiotics were gavaged to mice in the antibiotic and probiotic groups for23 days (including test days). In the last days of this period, Morris and Plus .tests were taken from the control and antibiotic groups

Probiotic course

After the antibiotic course, the groups identified for probiotics were divided into high dose andlow dose groups and the probiotics were orally gavaged for20 days (including test days) . Also in the final days of the course, Morris and Plus tests .were taken from the probiotic groups

Test days								examination Group	work explanation
31-32	30	25-29	24	22-23	17-21	12-16	1-11		
				■	■	■	■	آ	Antibiotic gavage
							■	P	
				■	■	■	■	ک	Normal saline gavage
■	■	■	■	■	■	■		P	Probiotic gavage
						■		آ	) MostTrain (and Morris test
						■		ک	
		■						P	
				■				آ	) MostTrain (and Test Plus
				■				ک	
■								P	

Table7 :Schedule and time of each stage of the experiment . (Groups, A : Antibiotics, C: Probiotics, K: Control)

Results

At the end of the antibiotic period, the feces of the mice of the antibiotic group changed toDiarrhea in contrast to which the mice of the control group had normal , feces. Return of feces of probiotic groups to normal was also observed in the last week. Graphs and statistical results from the analysis of the Morris water maze test in all four groups show that all the mice spent an average of the same time in the area of the maze where the platform was located before the probe day (without platform). However, in the case of the plus test, the heads of the probiotic groups spent on average less time than the other groups in the open arms of the maze, this time for the same antibiotic and control groups, as well as a significant difference between the two groups. There were no probiotics during this time. The Morris maze diagram is shown in Figure6 and the maze plus diagrams are shown in Figures7.1 and7.2 .

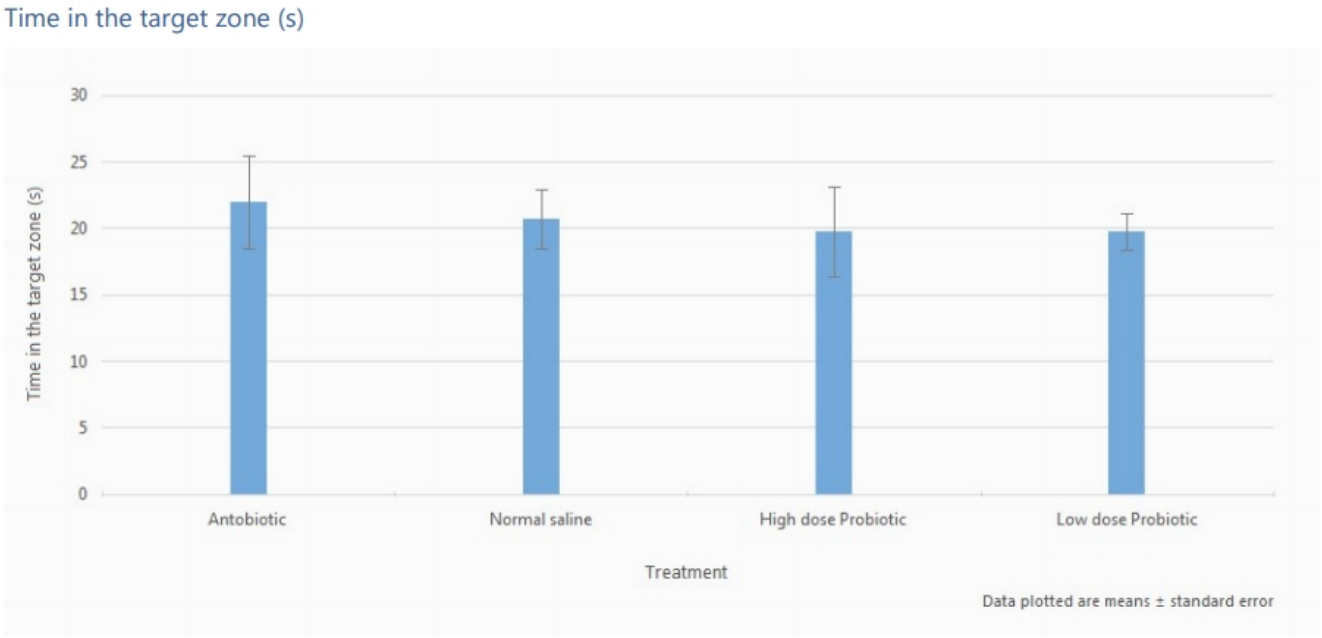


Figure 2. Time in the target zone. The data analysed has been limited in the following way: Treatment = Antobiotic, Normal saline, High dose Probiotic or Low dose Probiotic and Trial = Trial 1.

Figure6 Morris blue maze diagram :

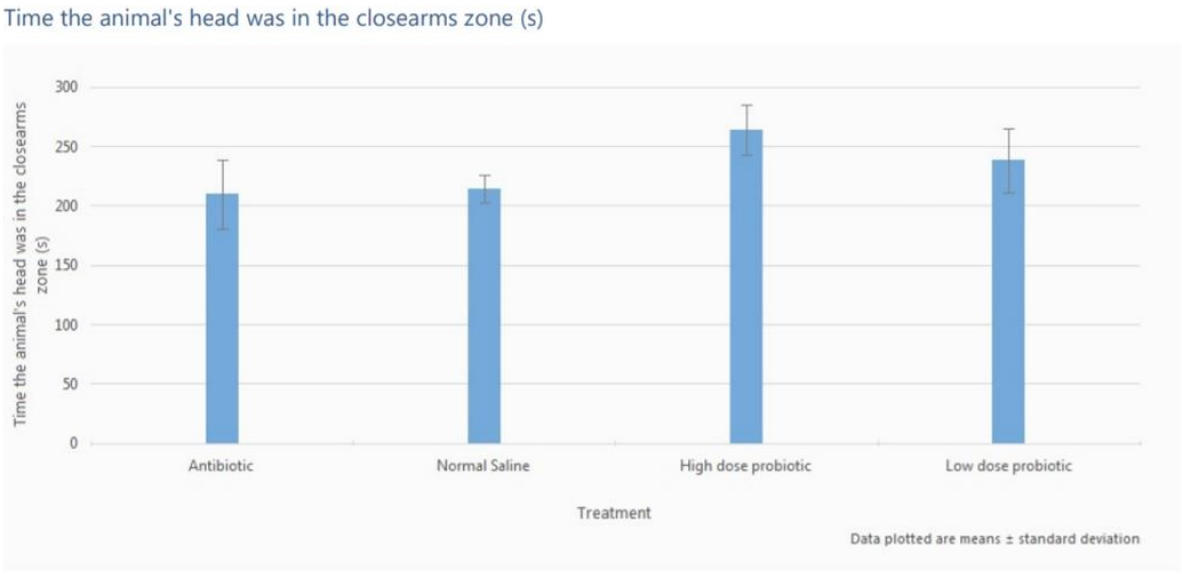


Figure 18. Time the animal's head was in the closearms zone. The data analysed has been limited in the following way: Treatment = Antibiotic, Normal Saline, High dose probiotic or Low dose probiotic and Trial = Trial 1.

Figure7.1 .Maze Plus diagram, when the heads of mice are spent in closed arms : (In seconds)

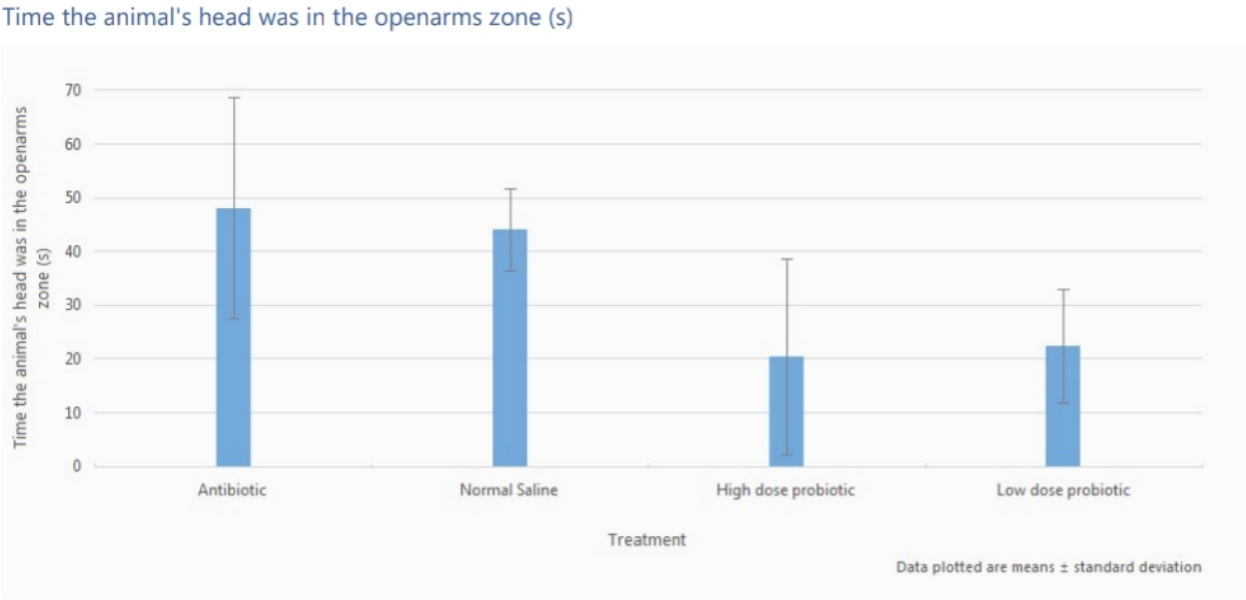


Figure 14. Time the animal's head was in the openarms zone. The data analysed has been limited in the following way: Treatment = Antibiotic, Normal Saline, High dose probiotic or Low dose probiotic and Trial = Trial 1.

Figure7.2 Maze Plus diagram, when mice spend their heads in open arms (in : seconds)

Analysis of results

Based on the above findings, it can be concluded that there is no significant difference in long-term spatial memory with the use of antibiotics in mice. A similar] .result has already been obtained for a course of antibiotic use in adult mice16 [Contrary to popular belief, taking antibiotics does not have a significant effect on anxiety, but taking probiotics **after a course of antibiotics** increases **anxiety** . In addition, according to the results, the use of probiotics **after a course of antibiotics does not have a significant** effect on long-term spatial memory. Of course, in other researchers, this result is similar for **A model of Alzheimer's rats** with a period of

] .probiotic use has been obtained29 [

one-way ANOVA, fixed factors test was used . for quantitative analysis of data p_value was less than 0.05 in all comparisons for the anxiety factor . Details of .statistical analysis of tests can be seen in the attached section

New hypotheses based on the results

Increased anxiety in mice may be due to the fact that as the bacterial population decreases and the gut microbiota is damaged by the antibiotic mixture, intestinal .permeability increases and probiotics enter the deeper layers of the intestinal wall Or, in other words, increase intestinal permeability by causing infection and inflammation; Therefore, with increasing permeability, more damage is done to the intestinal microbiota, which in turn increases anxiety in Mays. Of course, the correctness or incorrectness of the hypothesis can be determined by performing special microbiological tests on all four groups of mycelium and sampling epithelial .tissue using biopsy to measure intestinal barrier (intestinal permeability)

New solutions : Simulation of intestinal microbiota, a new way to better understand intestinal bacteria

For each simulation, we need a method. Also, each simulation is designed for a specific purpose and can not cover other purposes. For example, the method and method of simulating the bacterial population is different from the method and method of simulating the relationship between bacteria, and each requires its own separate equations. The simulation we are looking at here is an image simulation that aims to give a visual effect to the action and reactions between intestinal bacteria and cells in the intestinal wall. To achieve this goal, our model is the cellular automation model. Cellular automation is a model of discrete mathematics that has been studied in topics such as computability theory, mathematics, physics, complex adaptive systems, theoretical biology, and microstructures. Cellular automation is ,also referred to as cell spaces, automation automation, homogeneous structures .cellular structures, automation structures, and repetitive arrays

:materials and methods

:key To Achieve To Similar Making Visual Microbiota Intestine قدم های

1 Behavior کردن And Specified ها And Cell ها Category Classification Bacteria های ان ها

2 های Effective At Changes And Reaction های At Opinion Take Variable Microbiota Intestine

3 on foot Making Algorithm Similar Making, On Foundation Algorithm Main .Automatic Cellular

4 Automatically های Suitable To Equivalent Making Pattern های Find Plan .ها And Cell ها With Category Classification Bacteria

5 Order Suitable To state of primitive Automatically, Matching ریزی Plan .With Conditions Biological Real

Effective At This Similar Making To To General To Three Category های Bacteria (ها probiotics) Useful های damage, Bacteria بی های Harmful, Bacteria های Bacteria Can At Table می را ها ان To be. the list Accurate می divided Classification8 View .do

#	Bacteria	Permeability and safety effects
1	Clostridial sp	Restoration of blood serotonin and increased intestinal permeability
2	Lactobacillus	Reduce intestinal permeability by rearranging membrane proteins that are important in maintaining connections between epithelial cells and increasing mucus production
3	Escherichia coli	Similar to Lactobacillus
4	Bifidobacterium	Similar to Lactobacillus
5	Normal bacteria	Nutritional factors for mucosal and epithelial layers increase the proliferation ofcrypt cells .and villi

Restoration of blood andcolon serotonin under certain conditions	S pore-forming bacteria	6
Conversion of histidine to histamine	Lactobacillus reuteri	7
Increased concentration of tryptophan serotonin precursor in plasma	Bifidobacterium infantis	8
Increased anxiety	Campylobacter jejuni	9
Anti-anxiety but also requires intact vagus	Bifidobacterium longum	10
Anxiety probiotics	Lactobacillus rhamnosus	11

Table8

At System Nervous سزایی That the effect To ها Bacteria ترشّحی Also from Materials .Power name Win را می Fat Short Chain های And acid ها have, Liposaccharides Table)9 (

Description	Metabolites	#
They activate specific receptors in , epithelial cells, intestinal neurons sensory afferent neurons in the spine and	Liposaccharides)LPS -or neuro (active peptides	1

brain neurons, and affect the activity of the central nervous system and intestines . They also cause mood swings after an acute illness. Liposaccharides have been shown to cause depression and .anxiety in animal models		
Short-chain fatty acids can act as signaling molecules by binding to protein receptors. These molecules can increase intestinal permeability, involve the immune system and sympathetic nervous system, and cross the blood-brain barrier .to affect mood	Short chain fatty) acidsSCFAs (	2

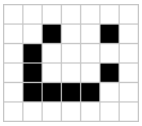
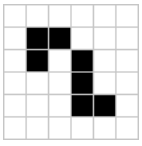
Table9

Algorithm Soft Software, The same Algorithm Play Life Conway And Highlife می آمیزی Color ها May Be, With This Difference That At This Similar Making, Cell Special Matching With های To be That sign Donor Existence Population From Cell می determination ان راسی های Any Cell On Basis Neighbor آمیزی Be. Color می Colors With ان های Be That Number Neighbor می Be, So the face That colorful Selection .The same Color the most Be

,Oh you شطنجی Materials, At pink That Any Home ترشّح To 3 cells Live Neighbor Home To ان ,Did not have Be ان را Had Be And Cell Live Oh you Intention Capture the next To های Be And At far می Item Opinion determination ترشّحی Title a matter

Slowly At pink That می Side Right Page (to Side Depth Wall Intestine) movement Be می Delete ترشّحی To Side Right Page Be, a matter ان Something Barrier Move .Is شده And perhaps From Through Feces From Body Out

Any population Cellular Also, Should To Behavior های To determination Pattern That At کرد Selection را And Pattern کرد population Accuracy ان Special To می From Self sign را population Cellular ان Automatically, Behavioral Similar To Give. (Table10 (

Image	Description	Equivalent cell population	Pattern	#
	The smallest vertical spacecraft and the second most common spacecraft after the glider	Harmful bacteria	Lightweight spaceship Lightweight spaceship (LWSS)	1
	The fish hook oreater is the first eater pattern to be discovered. This pattern was independently discovered in	Natural intestinal microbial flora	Eater 1	2

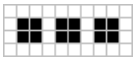
	1971 as the smallest asymmetricstill life by a number of life game . enthusiasts			
	Tri-block pattern consists of threeblock patterns and falls into the category ofpseudo still lives .	Intestinal wall	Tri-block	3

Table10

For the initial arrangement of the patterns, we must pay attention to the different layers of the intestinal wall and inside the intestine. According to studies, we can consider a ratio of ۴ for the lumen (intestinal tube) , ۵for the mucus layer and2 for thesubmucosa Finally, depending on the processing power of each computer, the . length and width can be determined, and the simulator determines the thickness of the layers according to the mentioned ratios and arranges the desired patterns on the screen (Figure8 .(

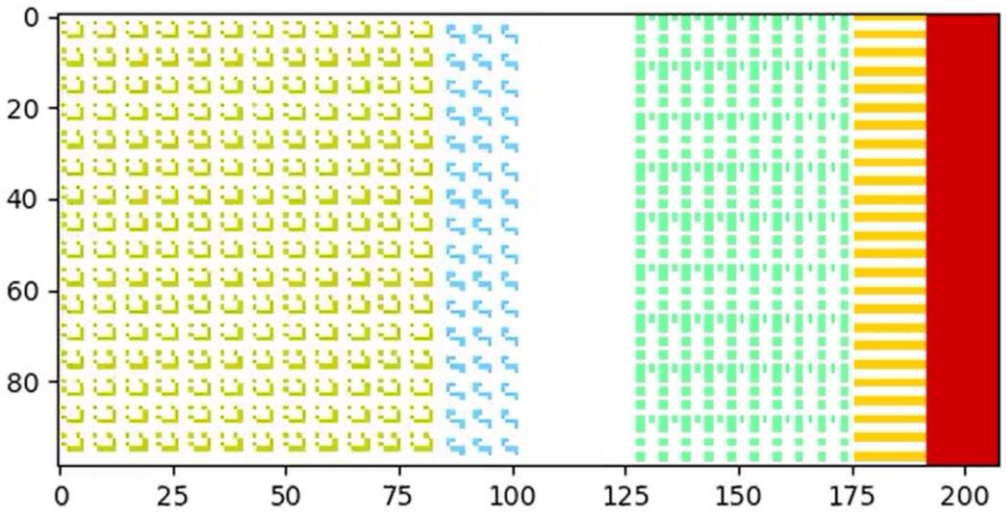


Figure8

: Results

In the end, with the production of this simulation program, we can get a better view of the interactions of the intestinal microbiota: (Figures9 and10 (

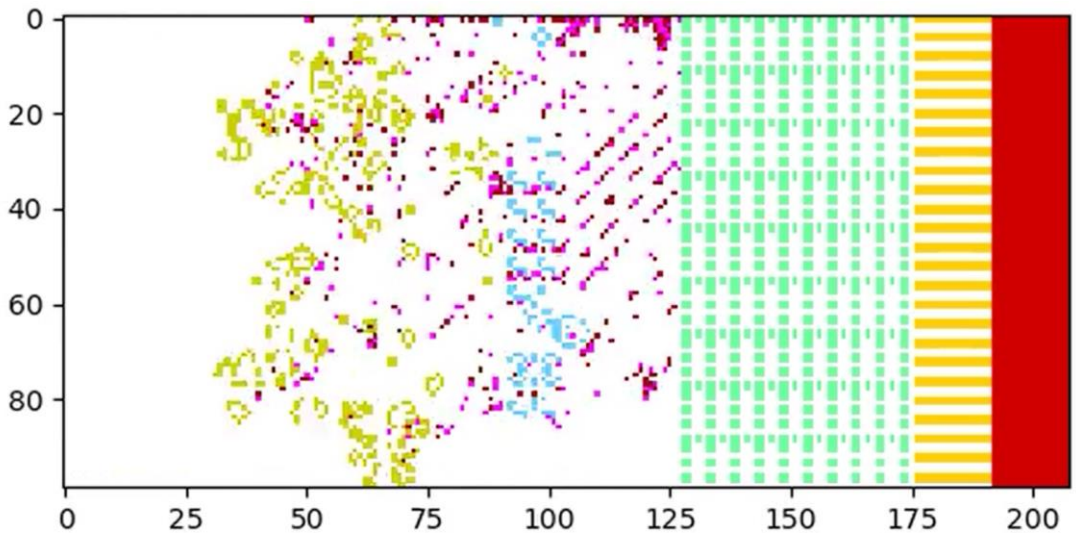


Figure9 :purple and pink: metabolites, mustard yellow: invasive bacteria, blue) :normal intestinal microbial flora, green: epithelial cells, yellow: nerve cells, red (blood flow

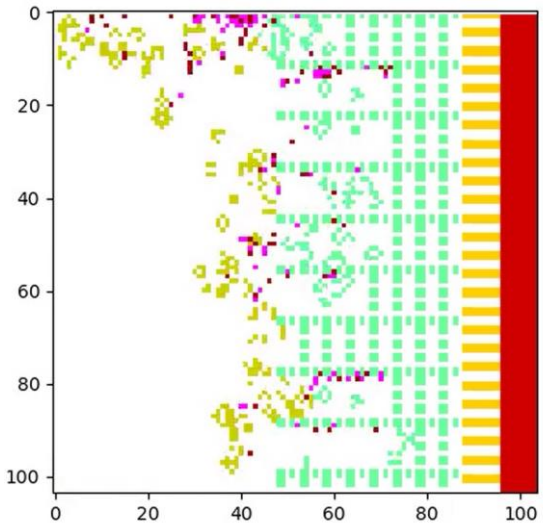


Figure10

: Conclusion

Our world is infinite, but cellular automation is often simulated on a finite network rather than an infinite network. Thus in the two-dimensional state, the universe must be quadrangular instead of an infinite plane. The problem with finite grids is how to manage cells in the corners. How they are managed will affect the values of all cells in the network. This can be managed by connecting the left and right corners of the rectangle to form a tube, then connecting the top and bottom corners of the tube to form a circle. Spaces related to other dimensions are managed in a similar way. So ,the problem of border boundaries in neighbors is solved. (Source: Wikipedia (Cellular Automation

Of course, it is debatable whether the microbiota simulation with cellular automata is true. As can be seen in nature, they follow a specific cellular automaton. For example, differential equations introduced by Turing to explain how hatches and .lines are created in animals are consistent with cellular automaton patterns

However, with the exception of complex mathematical methods, the interactions of cells and bacteria can be illustrated using simpler methods such as cellular automata with a little creativity. Of course, the purpose of this type of simulation was to better understand the behaviors of the gut microbiota. Note that this method is not .applicable to accurate and unpredictable assessments

Thank you

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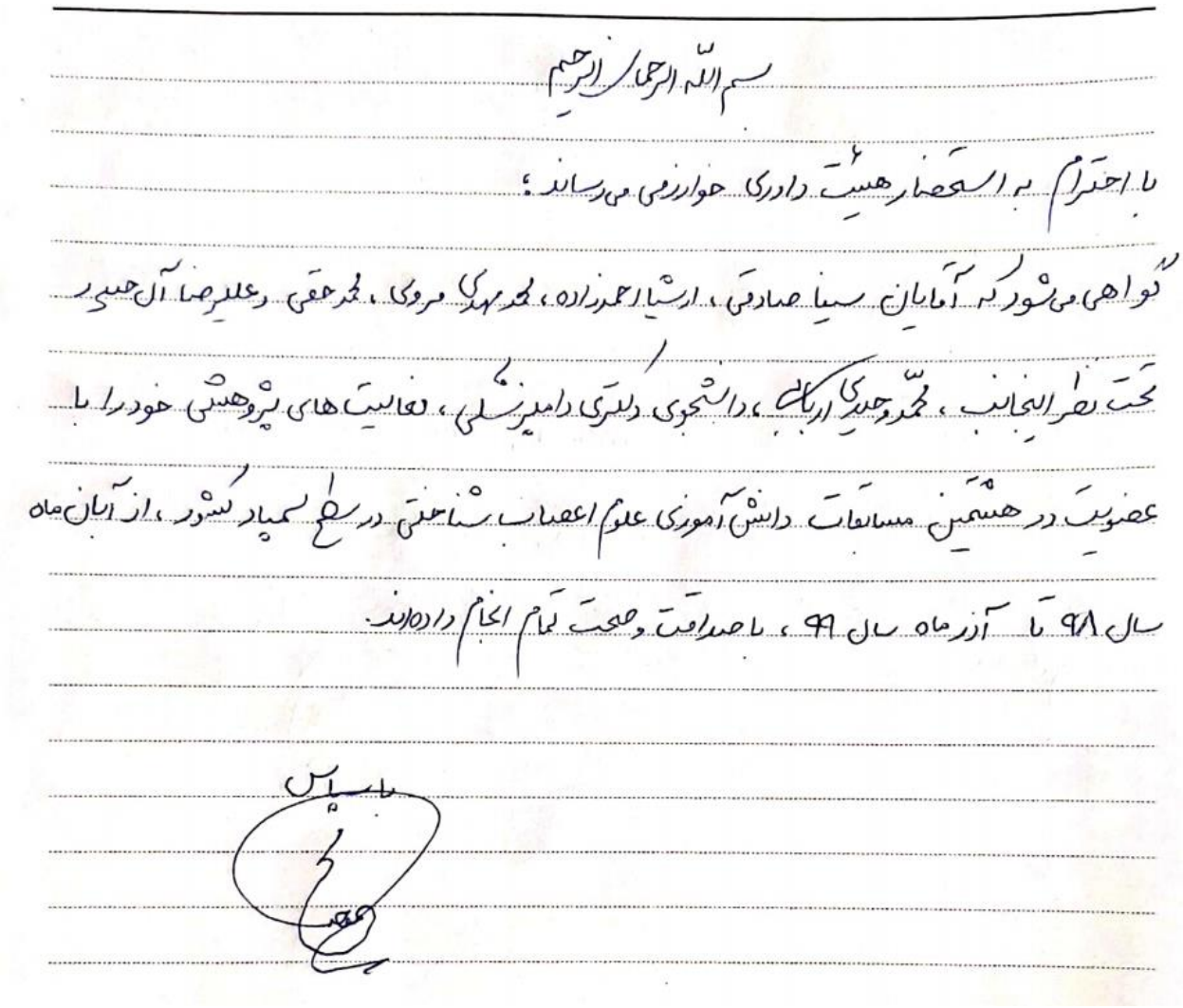

بسم الله الرحمن الرحیم

با احترام به استحضار هیئت داوری خوارزمی می رساند؛

گواهی می شود که آقایان سینا صادقی، ارشیا احمدزاده، محمدمهدی مروتی، محمدحقی و علیرضا آل حسینی تحت نظر اینجانب، محمد وحیدی اردکانی، دانشجوی دکتری دامپزشکی، فعالیت های پژوهشی خود را با عضویت در هشتمین مسابقات دانش آموزی علوم اعصاب شناختی در سطح کشور، از آبان ماه سال ۹۸ تا آذر ماه سال ۹۹، با صداقت و صحت تمام انجام داده اند.

Trial monitoring confirmation

Time in the target zone (s)

Treatment	*N*	*Mean*	*SE* (note 1)	*Data* (notes 2, 3)
Antobiotic	4	21.90	±3.46	26.2 (1.2), 20.8 (1.3), 12.6 (1.4), 28.0 (1.5)
Normal saline	4	20.63	±2.22	14.8 (2.1), 21.3 (2.2), 20.8 (2.3), 25.6 (2.4)
High dose Probiotic	5	19.70	±3.36	18.1 (3.1), 20.6 (3.2), 13.8 (3.3), 32.1 (3.4), 13.9 (3.5)
Low dose Probiotic	5	19.70	±1.34	17.8 (4.1), 22.6 (4.2), 23.0 (4.3), 18.9 (4.4), 16.2 (4.5)

ANOVA (note 4) : $F_{(3,14)} = 0.1418$ $p = 0.933$

Notes:

1. SE = Standard error.
2. The data analysed has been limited in the following way: Treatment = Antobiotic, Normal saline, High dose Probiotic or Low dose Probiotic and Trial = Trial 1.
3. The numbers in parentheses next to the data values are animal numbers - you can click these to access the animal's details report.
4. One-way ANOVA, fixed factor.

Morris test data: The amount of time a mouse has spent in an area of the maze where the platform was present

Time the animal's head was in the closearms zone (s)

Treatment	*N*	*Mean*	*SD* (note 1)	*Data* (notes 2, 3)
Antibiotic	4	209.15	±29.09	210.0 (1.2), 246.1 (1.3), 205.4 (1.4), 175.1 (1.5)
Normal Saline	4	213.70	±11.57	230.7 (2.1), 205.5 (2.2), 211.1 (2.3), 207.5 (2.4)
High dose probiotic	5	263.32	±20.89	268.7 (3.1), 239.4 (3.2), 243.8 (3.3), 277.9 (3.4), 286.8 (3.5)
Low dose probiotic	5	237.78	±27.25	199.7 (4.1), 259.8 (4.2), 267.9 (4.3), 234.6 (4.4), 226.9 (4.5)

ANOVA (notes 4, 5) : $F_{(3,14)} = 5.1651$ $p = 0.013$

Notes:

1. SD = Standard deviation.
2. The data analysed has been limited in the following way: Treatment = Antibiotic, Normal Saline, High dose probiotic or Low dose probiotic and Trial = Trial 1.
3. The numbers in parentheses next to the data values are animal numbers - you can click these to access the animal's details report.
4. One-way ANOVA, fixed factor.
5. $p \leq 0.05$ is highlighted in red.

. Test Plus data: How long Mice's head has been in closed arms

Time the animal's head was in the openarms zone (s)

Treatment	*N*	*Mean*	*SD* (note 1)	*Data* (notes 2, 3)
Antibiotic	4	48.05	±20.64	55.2 (1.2), 22.0 (1.3), 43.9 (1.4), 71.1 (1.5)
Normal Saline	4	43.97	±7.61	34.9 (2.1), 49.8 (2.2), 40.5 (2.3), 50.7 (2.4)
High dose probiotic	5	20.38	±18.17	23.4 (3.1), 49.6 (3.2), 18.0 (3.3), 7.3 (3.4), 3.6 (3.5)
Low dose probiotic	5	22.34	±10.55	40.3 (4.1), 15.0 (4.2), 14.6 (4.3), 22.4 (4.4), 19.4 (4.5)

ANOVA (notes 4, 5) : $F_{(3,14)} = 3.9791$ $p = 0.030$

Notes:

1. SD = Standard deviation.
2. The data analysed has been limited in the following way: Treatment = Antibiotic, Normal Saline, High dose probiotic or Low dose probiotic and Trial = Trial 1.
3. The numbers in parentheses next to the data values are animal numbers - you can click these to access the animal's details report.
4. One-way ANOVA, fixed factor.
5. $p \leq 0.05$ is highlighted in red.

.Test Plus data: How long the mice have spent in the open arms