

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

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

Dedication 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 of ♀ • Balb / c

mice into four groups \ Control . ♀ Antibiotics . ♀ Low dose probiotics . ♀ .

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

Persian keywords

,Intestinal microbiotaHPA axis ,Spatial memory, Anxiety, Vagus nerve, Antibiotic ,
Probiotic , Morris water maze, Suspended plus maze

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 systemCNS 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, important to identify the factors that upset the balance of the gut microbiota prevent them . In this study, an attempt has been made to understand the effects of these changes on spatial memory and anxiety by making changes in bacterial population of intestinal microbiota. To make changes to the gut microbiota of mice, a course of a mixture of four antibiotics was first 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 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 the field of cognitive neuroscience and have been used in valid scientific to study the cognitive factors in rodents. Due to the subtlety and specialization microbiology, accurate study of the composition of the gut microbiota is not possible and requires advanced equipment and technologies that are only available in certain countries. Therefore, one of the important challenges in studies is the proper and accurate evaluation of the composition of the

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 [1]. antibiotics do not affect spatial memory by disrupting the gut microbiota [2]. 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 by microorganisms 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 and actinobacteria. 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 genome DNA 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 the HPA 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 bacteria

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. Gangrene 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

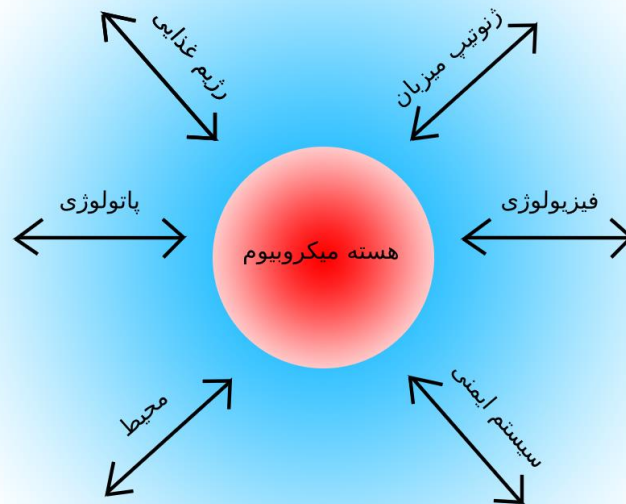


Figure \ Factors affecting the intestinal microbiota :

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 injury³ . [

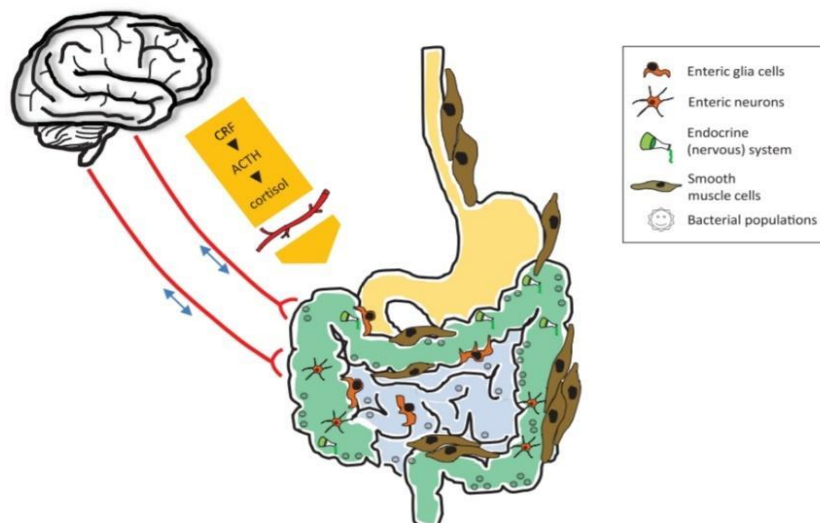
Intestinal microbiota is also influential in cognitive diseases such as autism, and scientists in recent years have concluded that autism is one of the diseases associated with altered intestinal microbiota. In fact, scientists have discovered that a decrease in the population of *Akkermansia muciniphila* and *Bifidobacterium* 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 with HPA axis

HPA stands for Hypothalamus, 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 the HPA axis is a group of microbes that are in our gut and are called "Gut 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 releasedHPAThey can also regulate the gut microbiota. According to research, colonization ofBifidobacterium 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. Figure 2 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 secreteACTH . ACTH affects the adrenal gland and the adrenal gland begins to secrete cortisol, which affects the gastrointestinal tract

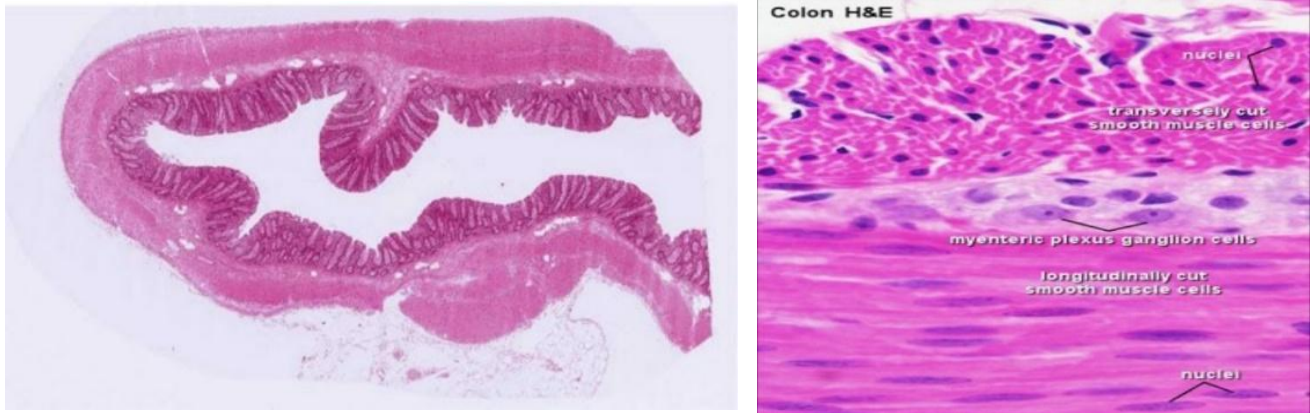


. and intestinal microbiome

Figure 2 Relation of microbiome to HPA axis

How the nervous system communicates with the microbiome

In Figures 3.1 and 3.2 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 and 3.2 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 leptin [11]

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

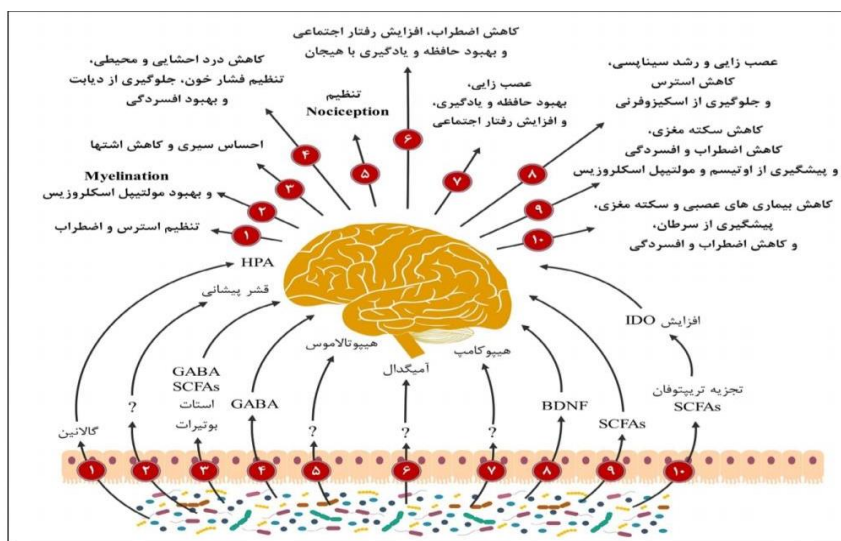


Figure4

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 plus Elevated 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 for 14 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 day onwards the probiotic group was not given a mixture of antibiotics and the probiotic course for them started from this day. High dose 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. Table 1 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

Table 1 | Bacteria and neurotransmitters secreted by them [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 norepinephrine, dopamine, 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 Table)3 .(

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
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Table 3 | Specified doses for antibiotics

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 are Comflor and BioFem .

Table 4 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

Bio Fem	Bifidobacterium longum
	Bifidobacterium breve
	Bifidobacterium Infantis
	Streptococcus thermophilus
	Lactobacillus Rhamnosus
	Lactobacillus Reuteri

Table 5

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

Table 5 Specified doses for probiotics, according to the prescription of each drug :

Finally, you can get an overview of the groups studied in Table 6 you have. Each group contains 4 mice

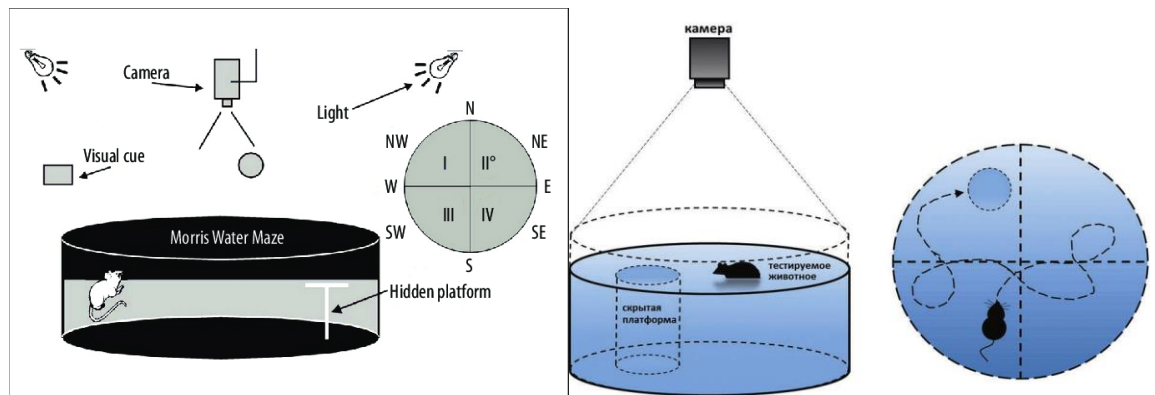
Probiotic dose	probiotic	Antibiotics	group
-	X	X	control group
-	X	✓	Antibiotic group
Low dose	✓	✓	Le Dos probiotic group
High dose	✓	✓	Dosage probiotics group

Table 6 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 and Elevated Plus maze. At the end of the antibiotic and probiotic courses, all the mice, Morris water maze and Elevated 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 and Y, this test does not require a hunger test for mice,



Figures 5.1 and 5.2 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

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 and Elevated plus maze .

Antibiotic course

Mixtures of antibiotics were gavaged to mice in the antibiotic and probiotic groups for 23 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 and low dose groups and the probiotics were orally gavaged for 23 days (including test days) . Also in the final days of the course, Morris and Plus tests were taken from the probiotic groups

Test days	exami	work
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۳۱-۳۲	۳۰	۲۵-۲۹	۲۴	۲۲- ۲۳	۱۷-۲۱	۱۲-۱۶	۱-۱۱	nation Group	explanation
								Ā	Antibiotic gavage
								P	
								κ	Normal saline gavage
								P	Probiotic gavage
								Ā) MostTrain (and Morris test
								κ	
								P	
								Ā) MostTrain (and Test Plus
								κ	
								P	

Tablev :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 to Diarrhea 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 Figure 6 and the maze plus diagrams are shown in Figures 7.1 and 7.2 .

Time in the target zone (s)

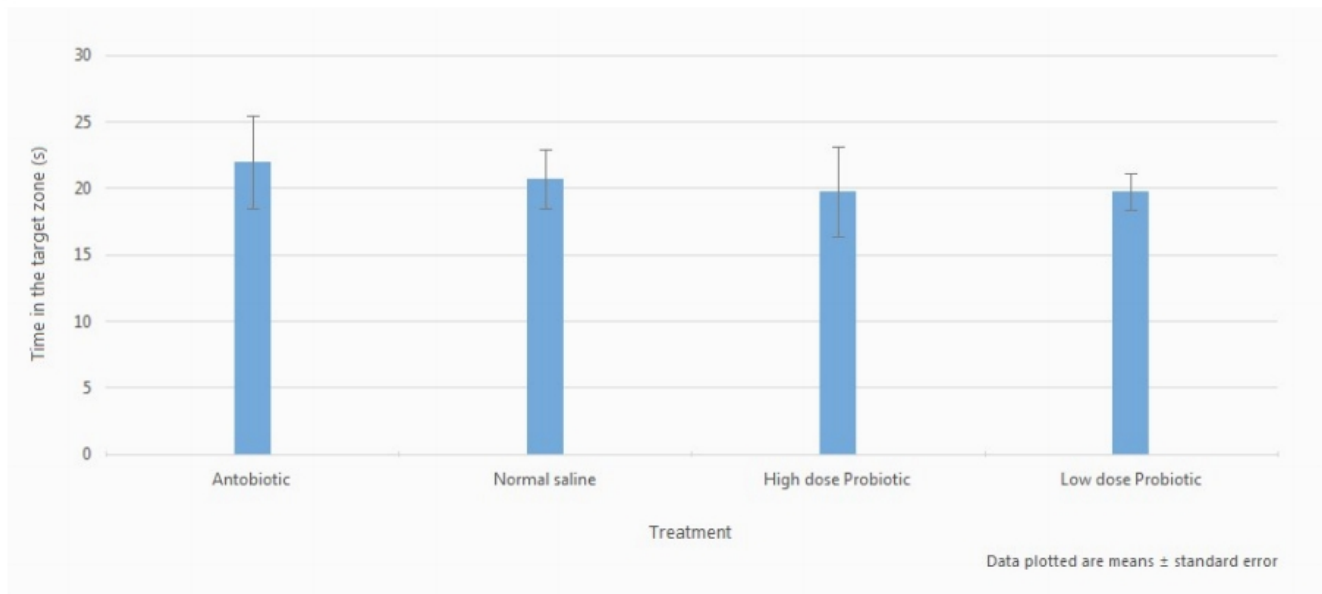


Figure 2. Time in the target zone. The data analysed has been limited in the following way: Treatment = Antibiotic, Normal saline, High dose Probiotic or Low dose Probiotic and Trial = Trial 1.

Figure6 Morris blue maze diagram :

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

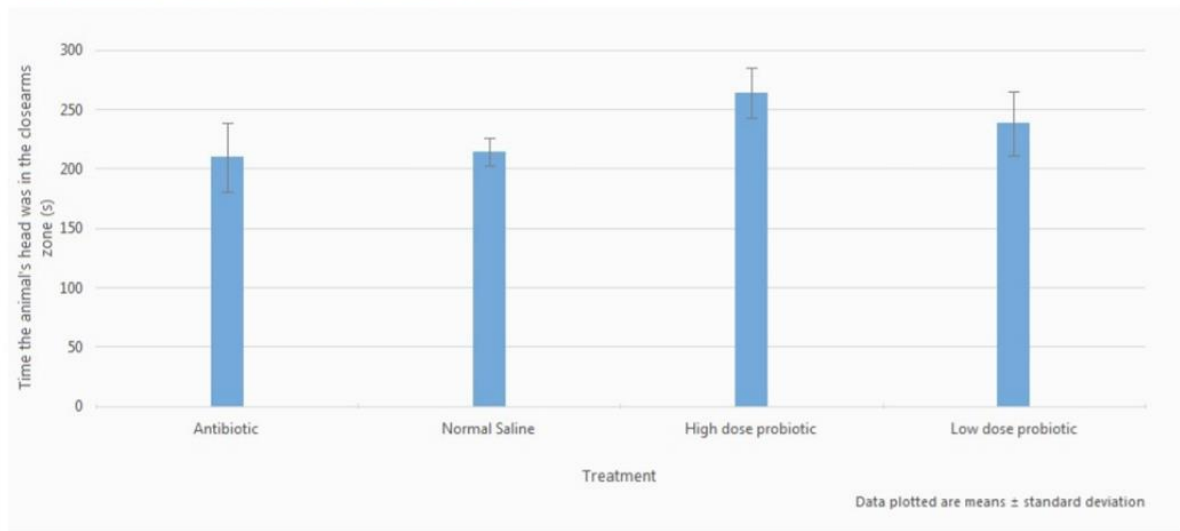


Figure 18. Time the animal's head was in the closearms zone. The data analysed has been limited in the following way: Treatment = Antibiotic, Normal Saline, High dose probiotic or Low dose probiotic and Trial = Trial 1.

Figure 19. Maze Plus diagram, when the heads of mice are spent in closed arms :
(In seconds)

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

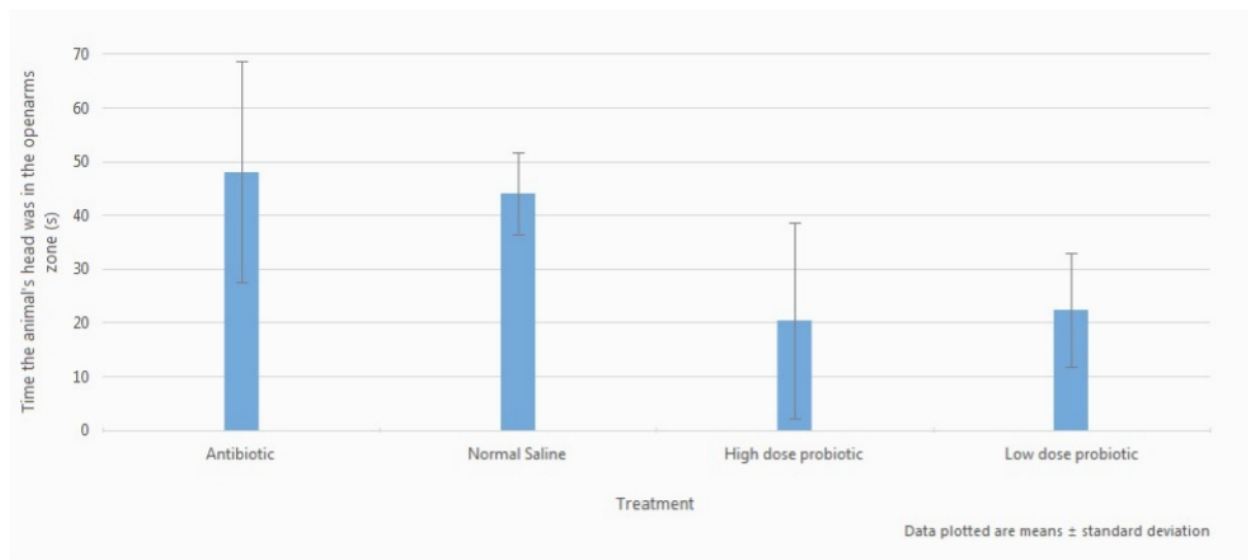


Figure 14. Time the animal's head was in the openarms zone. The data analysed has been limited in the following way: Treatment = Antibiotic, Normal Saline, High dose probiotic or Low dose probiotic and Trial = Trial 1.

Figure 14. 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 mice [16]. 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

materials and methods

قدم های key To Achieve To Similar Making Visual Microbiota Intestine

- 1 Behavior And Specified ها Cell ها Category Classification Bacteria
های ان ها
- 2 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 Suitable To Equivalent Making Pattern های Find Plan
And Cell ها With Category Classification Bacteria
- 5 Order Suitable To state of primitive Automatically, Matching With ریزی Plan
Conditions Biological Real

Effective At This Similar Making To To General To Three Category های Bacteria

Bacteria های Bacteria Harmful, Bacteria های بی damage, Bacteria های Useful (probiotics) ها

Can At Table می To be. the list Accurate divided Classification^۸.View do

Permeability and safety effects	Bacteria	#
Restoration of blood serotonin and increased intestinal permeability	Clostridial sp	١
Reduce intestinal permeability by rearranging membrane proteins that are important in maintaining connections between epithelial cells and increasing mucus production	Lactobacillus	٢
Similar to Lactobacillus	Escherichia coli	٣
Similar to Lactobacillus	Bifidobacterium	٤
Nutritional factors for mucosal and epithelial layers increase the proliferation of crypt cells and villi	Normal bacteria	٥
Restoration of blood and colon serotonin under certain conditions	S pore-forming bacteria	٦
Conversion of histidine to histamine	Lactobacillus reuteri	٧

Increased concentration of tryptophan serotonin precursor in plasma	Bifidobacterium infantis	۸
Increased anxiety	Campylobacter jejuni	۹
Anti-anxiety but also requires intact vagus	Bifidobacterium longum	۱۰
Anxiety probiotics	Lactobacillus rhamnosus	۱۱

Table8

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

۹ (

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

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 (۲

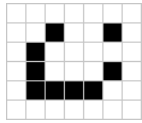
Table9

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

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,Oh you شطنجی Materials, At pink That Any Home To ترشح cells Live Neighbor
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Slowly At pink That می Side Right Page (to Side Depth Wall Intestine) movement
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Table)۱۰ (

Image	Description	Equival t cell populati n	Pattern	#
	The smallest vertical spacecraft and the second	Harmful bacteria	Lightweight spaceship	۱

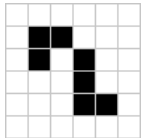

	most common spacecraft after the glider		Lightweight spaceship (LWSS)	
	The fish hook oreater is the first eater pattern to be discovered. This pattern was independently discovered in ۱۹۷۱ as the smallest asymmetric still life by a number of life game . enthusiasts	Natural intestinal microbial flora	Eater ۱	۲
	Tri-block pattern consists of threeblock patterns and falls into the category ofpseudo still lives .	Intestinal wall	Tri-block	۳

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 ϵ for the lumen (intestinal tube) , δ for the mucus layer and γ

for the submucosa. 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 (Figure 8).

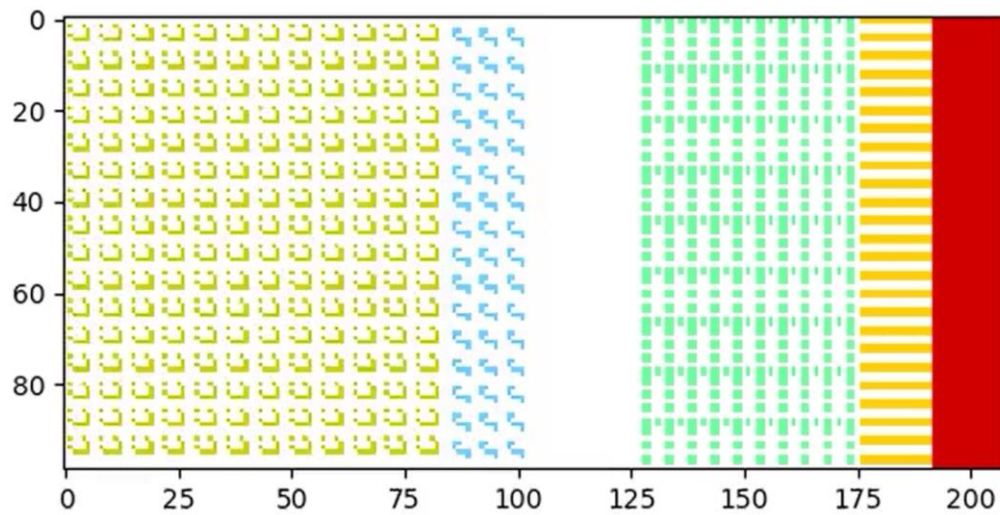


Figure 8

: Results

In the end, with the production of this simulation program, we can get a better view of the interactions of the intestinal microbiota: (Figures 9 and 10).

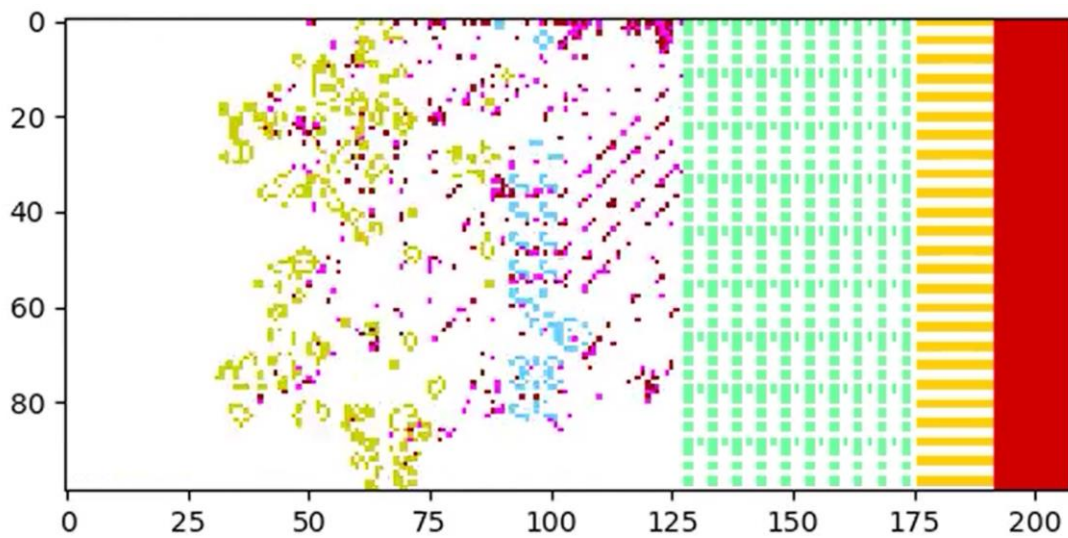


Figure 9 :purple and pink: metabolites, mustard yellow: invasive bacteria, blue) :normal intestinal microbial flora, green: epithelial cells, yellow: nerve cells, red (blood flow

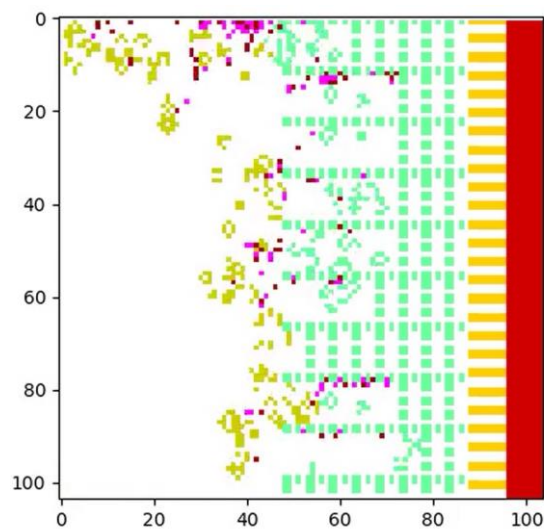


Figure10

: 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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References

- 1 Neurotransmitter modulation by the gut microbiota / Philip Strandwitz / Brain Res / 2018 Aug 15
- 2 Antibiotic Resistance and Plasmid DNA Contents of Streptococcus thermophilus Strains Isolated from Turkish Yogurts / B elma A slim , Y avuz B eyatli / Turk J Vet Anim Sci / 16.08.2001
- 3 Antimicrobial susceptibility patterns of *E. coli* from clinical sources in

- northeast Ethiopia / M Kibret and B Abera / Afr Health Sci / 2011 Aug
- 4 In vitro susceptibility of *Bacillus* spp. to selected antimicrobial agents / DJ Weber, SM Saviteer, WA Rutala, and CA Thomann / Antimicrob Agents Chemother / 1988 May
 - 5 Antimicrobial Susceptibility of *Bacillus* Strains Isolated from Primary Starters for African Traditional Bread Production and Characterization of the Bacitracin Operon and Bacitracin Biosynthesis / David B. Adimpong, Kim I. Sørensen, Line Thorsen, Birgitte Stuer-Lauridsen, Warda S. Abdelgadir, Dennis S. Nielsen, Patrick MF Derkx, and Lene Jespersen / Appl Environ Microbiol / 2012 Nov
 - 6 Antimicrobial susceptibility of Bifidobacterium strains from humans, animals and probiotic products / Koenraad Van Hoorde, Liesbeth Masco, Evie De Brandt, Jean Swings / Journal of Antimicrobial Chemotherapy / August 2006
 - 7 Antimicrobial Susceptibility of *Bacillus* Strains Isolated from Primary Starters for African Traditional Bread Production and Characterization of the Bacitracin Operon and Bacitracin Biosynthesis / David B. Adimpong, Kim I. Sørensen, Line Thorsen, Birgitte Stuer-Lauridsen, Warda S. Abdelgadir, Dennis S. Nielsen, Patrick MF Derkx , and Lene Jespersen / Appl Environ Microbiol / 2012 Nov
 - 8 Medication Usage Chart for Rat Respiratory Illnesses and Other Conditions / Mary Ann Isaksen and Daryl Mabley, DVM (Revised by M. Isaksen) / www.rmca.org / July, August 2000

9 <http://www.siumed.edu/~dking2/erg/gicells.htm>

10 Junqueira's Basic Histology: Text and Atlas, Thirteenth Edition / Anthony Mescher

11 The microbiome: A key regulator of stress and neuroinflammation. Neurobiol Stress / Rea K, Dinan TG, Cryan JF / Neurobiol Stress / 2016

12 The Gut Microbiome and the Brain / Leo Galland / J Med Food / 2014 Dec

13 Susceptibility testing of *Bacillus* species / JM Andrews, R. Wise / *Antimicrobial Chemotherapy* / June 2002

14 Antibiotic Resistance-Susceptibility Profiles of *Streptococcus thermophilus* Isolated from Raw Milk and Genome Analysis of the Genetic Basis of Acquired Resistances / Ana B. Flórez and Baltasar Mayo / Front Microbiol / 2017

15 Disruption of the Gut Ecosystem by Antibiotics / Mi Young Yoon ¹ and Sang Sun Yoon / Yonsei Med J / 2018 Jan

16 Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication / Esther E.Fröhlich, Aitak Farzi, Raphaela Mayerhofer, Florian Reichmann , Angela Jačan , Bernhard

Wagner , Erwin Zinser , Natalie Bordag , Christoph Magnes , Eleonore Fröhlich , Karl Kashofer , Gregor Gorkiewicz fgh , PeterHolzer / Elsilver / August 2016

17 The influence of antibiotics and dietary components on gut microbiota / Ruth K. Dudek-Wicher, Adam Junka, and Marzenna Bartoszewicz / Prz Gastroenterol / 2018

18 Antibiotics and the gut microbiota / Sheetal R. Modi, James J. Collins, and David A. Relman_ / The Journal of Clinical Investigation / October 1, 2014

19 Collecting Fecal Samples for Microbiome Analyzes in Epidemiology Studies / Rashmi Sinha, Jun Chen, Amnon Amir, Emily Vogtmann, Jianxin Shi, Kristin S. Inman, Roberto Flores, Joshua Sampson, Rob Knight and Nicholas Chia / AACR / February 2016

20 Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature / Sabina Fijan / International Journal of Environmental Research and Public Health / 2014

21 Behavioral phenotyping of mice in pharmacological and toxicological research / Tim Karl, Reinhard Pabst, and Stephan Vonhorsten / Elsilver / 2003

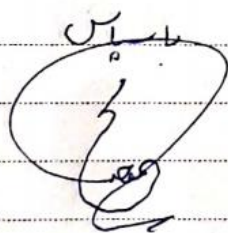
22 The effect of probiotics mixture on learning and spatial memory in kindled rats / Samaneh Bagheri , Azhdar Heidari , Azam Alinaghypour ,

Seyyed Mojtaba Banitaba , Mahmoud Salami / Feyz / 2018

- 23 The microbiota – gut – brain axis: neurobehavioral correlates, health and sociality / Augusto J. Montiel-Castro, Rina M. González-Cervantes, Gabriela Bravo-Ruiseco and Gustavo Pacheco-López / Front. Integr. Neurosci. / 2013
- 24 Neurotransmitter modulation by the gut microbiota / Philip Strandwitz / Brain Res / 2018
- 25 Probiotics and therapeutic applications / Moin Hamidi Hesari / Miad / Bani Tarfi / Laboratory and Diagnosis Quarterly1397
- 26 Antibiotics, microbiota, and immune defense / Carles Ubeda and Eric G. Pamer / Trends Immunol / 2012
- 27 A review of behavioral tests for memory and learning assessment in / rats / Fahimeh Zavari, Fariba Karimzadeh / Shafae Khatam2017
- 28 The Brain-Gut-Microbiome Axis / Clair R. Martin, Vadim Osadchiy, Amir Kalani, and Emeran A. Mayer / Cellular and molecular gastroenterology and hepatology / 2018
- 29 The Effects of Probiotic *Lactobacillus* and *Bifidobacterium* Strains on Memory and Learning Behavior, Long-Term Potentiation (LTP), and Some Biochemical Parameters in β -Amyloid-Induced Rat's Model of Alzheimer's Disease / Zahra Rezaeiasl, Mahmoud Salami, Gholamreza Food Sci. / 2019

the attachment

بسم الله الرحمن الرحيم
 با احترام به استحضار هست. دادری حواری می رساند؛
 خواهی می شود که آقایان سید صادقی، ارشیا احمدزاده، محمد مهدی مروتی، محمد عقی و علیرضا آل حسینی
 تحت نظر انجانب، محمد حیدر ارباب، دانشجوی دلیری دامپزشکی، فعالیت های پژوهشی خود را با
 عضویت در هشتین مسابقات دانش آموزی علوم اعصاب شناختی در سطح کمپاد کشور، از آبان ماه
 سال ۹۸ تا آذر ماه سال ۹۹، با صداقت و صحت تمام انجام داده اند.

باسلام


Trial monitoring confirmation

Time in the target zone (s)

<i>Treatment</i>	<i>N</i>	<i>Mean</i>	<i>SE</i> (note 1)	<i>Data</i> (notes 2, 3)
Antibiotic	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 = 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.

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)

<i>Treatment</i>	<i>N</i>	<i>Mean</i>	<i>SD</i> (note 1)	<i>Data</i> (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)

<i>Treatment</i>	<i>N</i>	<i>Mean</i>	<i>SD</i> (note 1)	<i>Data</i> (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