



University of Camerino

MASTER DEGREE IN COMPUTER SCIENCE

Course: Multiagent Systems Lab

Gut dysbiosis leads to Parkinson Disease through misfolding of α -synuclein

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Abstract

Disorders characterized by α -synuclein accumulation and Lewy body formation are collectively known as Lewy body diseases, of which one is Parkinson's Disease. The molecular mechanism through which α -synuclein abnormally accumulates and contributes to neurodegeneration in these disorders remains unknown. Some studies have demonstrated the presence of α -synuclein in the gut long before the appearance of the first phase of Parkinson's disease. Recently, it has been recognized that the brain-gut axis interactions are significantly modulated by the gut microbiota via immunological, neuroendocrine and direct neural mechanism. Disregulation of the brain-gut axis microbiota in PD may be associated with gastrointestinal manifestation frequently preceding motor symptoms, as well as with the pathogenesis of PD itself, supporting the hypothesis that the pathological process is spread from the gut to the brain. Excessive stimulation of the innate immune system resulting from gut dysbiosis and/or small intestinal bacterial overgrowth and increased intestinal permeability may induce systemic inflammation, while activation of enteric neurons and enteric glial cells may contribute to the initiation of α -synuclein misfolding. The intent of our work is to represent how gut dysbiosis can cause an overproduction of misfolded α -synuclein proteins in the gut and how they reach the brain passing first through the ENS (enteric nervous system) and then through the vagus nerve, causing Parkinson's.

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1. Introduction

1.1 Parkinson's Disease

Parkinson's disease (PD) is a progressive neurological disorder that primarily affects movement. It is characterized by a gradual loss of dopamine-producing cells in the brain, particularly in a region called the substantia nigra. Dopamine is a neurotransmitter that plays a crucial role in coordinating smooth and controlled movements. The exact cause of Parkinson's disease is not well understood, and it likely involves a combination of genetic and environmental factors. Age is a significant risk factor, with the majority of cases diagnosed in individuals over the age of 60. However, early-onset Parkinson's can occur in younger individuals.

There is currently no cure for Parkinson's disease, but there are medications and therapies that can help manage symptoms and improve the quality of life for individuals with the condition. In some cases, surgical interventions like deep brain stimulation (DBS) may be considered to alleviate symptoms. Parkinson's disease is a chronic condition, and its progression varies from person to person. Ongoing research is focused on understanding the underlying mechanisms of the disease and developing new treatments.

1.1.1 Some main causes

There are some potential causes like:

- **Neuroinflammation:** chronic inflammation in the brain may contribute to the degeneration of dopamine-producing neurons. This inflammation could be triggered by various factors, including infections or the body's immune response to certain stimuli.
- **Mitochondrial dysfunction:** dysfunction in the mitochondria, the energy-producing organelles within cells, has been implicated in Parkinson's disease. Mitochondrial dysfunction may contribute to oxidative stress, which can damage cells over time.
- **α -synuclein accumulation:** one of the pathological hallmarks of Parkinson's disease is the accumulation of α -synuclein proteins in the form of Lewy bodies within neurons. The aggregation of α -synuclein is thought to play a role in the degeneration of dopamine-producing neurons, although the exact mechanisms are not fully understood.

1.2 Central Nervous system

The central nervous system (CNS) is the master control center of the body, responsible for coordinating and regulating all voluntary and involuntary actions. Comprised of the brain and the spinal cord, the CNS serves as the epicenter for processing sensory information, initiating responses, and orchestrating complex behaviors.

The brain, often described as the most complex organ in the human body, is divided into several regions, each with specialized functions. These regions include the cerebrum, which controls conscious thought, sensory perception, and voluntary movement; the cerebellum, which coordinates motor movements and balance; the brainstem, which regulates basic involuntary functions such as heartbeat and breathing; and the limbic system, which governs emotions and memory.

The spinal cord, a long, slender bundle of nerves extending from the base of the brain down the vertebral column, acts as a conduit for transmitting signals between the brain and the peripheral nervous system (PNS). It relays sensory information from the body to the brain and conveys motor commands from the brain to the muscles and organs, enabling movement and regulating bodily functions.

Together, the brain and spinal cord form a complex network of neurons and support cells, known as glia, that communicate through electrical and chemical signals. This intricate communication system allows for the integration of sensory inputs, the generation of appropriate responses, and the maintenance of homeostasis—ensuring that the body functions optimally in response to internal and external stimuli.

The CNS plays a fundamental role in virtually every aspect of human experience, from basic survival instincts to higher cognitive processes like reasoning, creativity, and self-awareness. Its intricate structure and unparalleled capabilities underscore its significance as the ultimate control center of the human body.

1.2.1 Neuron

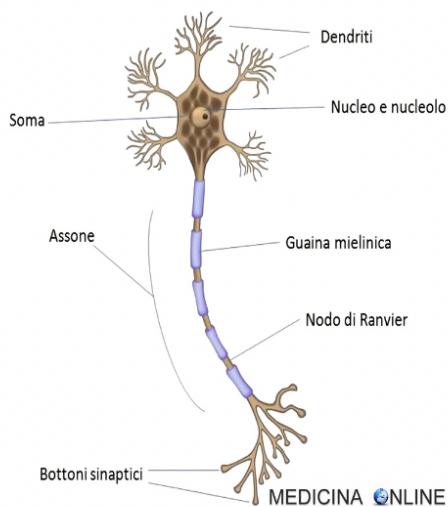


Figure 1.1: Image of a Neuron Cell

A neuron, or nerve cell, is the fundamental unit of the nervous system responsible for

transmitting information throughout the body. Structurally, neurons consist of three main parts: the cell body (soma), dendrites, and axon.

The cell body contains the nucleus and other organelles necessary for the neuron's metabolic functions. Dendrites are branched extensions that receive signals from other neurons or sensory receptors, while the axon is a long, slender projection that carries electrical impulses away from the cell body toward other neurons, muscles, or glands.

Neurons communicate with each other through a process called neurotransmission. When a neuron receives signals from other neurons or sensory receptors via its dendrites, these signals are integrated in the cell body. If the combined signals are strong enough to surpass a certain threshold, the neuron generates an electrical impulse known as an action potential.

The action potential travels down the axon toward the axon terminals, where it triggers the release of neurotransmitters into the synaptic cleft, the small gap between the axon terminal of one neuron and the dendrite or cell body of another neuron. Neurotransmitters bind to receptors on the postsynaptic membrane of the receiving neuron, leading to changes in its electrical activity. This can either excite or inhibit the receiving neuron, influencing whether it will generate its own action potential.

Overall, neurons play a crucial role in the transmission and processing of information within the nervous system, enabling various bodily functions and behaviors. They form complex networks that underpin sensory perception, motor control, cognition, and other essential aspects of human life.

1.2.2 α -synuclein

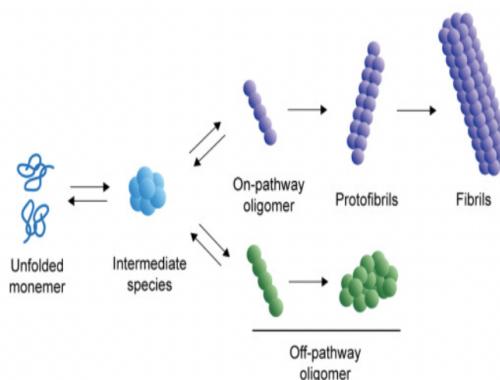


Figure 1.2: Aggregation of misfolding proteins

α -synuclein is a protein that is abundant in the human brain, particularly in the neurons. It plays a role in neurotransmitter release and is involved in the regulation of synaptic function. In its normal form, α -synuclein is soluble and exists as a monomer, which means it is made up of a single peptide chain.

However, α -synuclein has gained significant attention due to its association with several neurodegenerative disorders, particularly Parkinson's disease (PD). In PD, α -synuclein aggregates abnormally, forming insoluble clumps of protein called Lewy bodies. These aggregates are believed to contribute to the progressive degeneration of neurons, particularly those involved in movement control.

The exact mechanism by which α -synuclein aggregation leads to neuronal dysfunction and death is not fully understood, but it is thought to involve disruptions in cellular processes, such as protein trafficking, mitochondrial function, and synaptic transmission.

Research into α -synuclein is ongoing, with scientists exploring ways to prevent or reverse its abnormal aggregation as a potential therapeutic strategy for Parkinson's disease and related disorders. Understanding the role of alpha-synuclein in neurodegeneration may also shed light on the underlying mechanisms of other neurodegenerative diseases.

1.3 Gut microbiota

Microbiota refers to the entire population of microorganism that colonizes a particular location. It includes not just bacteria, but also other microbes such as fungi, archaea, viruses, and protozoans.

Significant interest has evolved on the gut microbiota in the recent years as the gut microbiota has been associated with a large array of human diseases. It has been speculated for a long time that the gut microbiota bears a significant functional role in maintaining the gut healthy. From an immunological perspective, microorganism are viewed as pathogens by the host immune system that recognizes and eliminates them. However, the majority of the gut bacteria are non-pathogenic and co-habit with enterocytes in a symbiotic relationship. The gut microbes predominantly aid in nutrient metabolism, drug metabolism, prevention of colonization pathogenic microorganisms and in intestinal barrier function. At the same time, the immune system has evolved to live in a collaborative relationship with the healthy microbiota, while serving its function to fight off invasive pathogenic microorganism.

1.3.1 Gut Dysbiosis

The most typical consequences of dysbiosis are a decrease in the diversity of the microbiota, a loss of beneficial microbiota and an overgrowth of harmful microbiota. Dysbiosis can be caused by host-specific factors such as genetic background, health status (ongoing infections, inflammations) and lifestyle habits or more importantly environmental factors such as diet (high sugar, low fibre), xenobiotics (antibiotics, drugs, food additives), and hygiene.

Profound changes in the gut bacterial and fungal microbiota can be rapidly achieved by changes in macro-nutrients. These changes have significant physiological consequences as, for example, diets rich in simple sugars disrupt the intestinal barrier, trigger intestinal inflammation, and negatively affect host metabolism. However, in most cases, interaction between diet and microbiota are necessary for these deleterious effects, as they do not occur in the absence of the gut microbiota. The effect of food additives on the gut microbiota has long been overlooked, but recently several groups have published data showing that some human gut microbiota are very sensitive to preservatives, and also that exposure to common food preservatives promotes overgrowth of proteobacteria. Other categories of additives have also been shown to have negative effects on human health.

The disruption of the gut microbiota ecosystem has many consequences, for example the weakening of the gut barrier and an imbalance of the immune and metabolic system,

particularly between regulatory and pro-inflammatory immune cells. The integrity of the intestinal wall could be compromised by acetaldehyde produced by microbiota from exogenous or endogenous ethanol, direct mucolytic activity and other mechanisms. Effects on the host metabolic system, particularly glucose and lipid metabolism, are mediated by changes in bile acid composition, production of short-chain fatty acids from dietary fibre, conversion of choline to trimethylamine and many other.

Aging itself is not considered a disease, but is a critical risk factor for a wide array of disorders. Aging comes with an increased risk of infection and chronic low-grade inflammation known as "inflamm-aging". This phenotype is mainly due to the physiological changes that result in frailty and immunosenescence, which leads to increased infections in the elderly population. Changes in microbiota diversity have been observed over the lifespan of human and during the progression of aging. Many studies have shown dramatic changes within the microbiome composition and gut structure in the elderly population.

1.3.2 Lipopolysaccharide

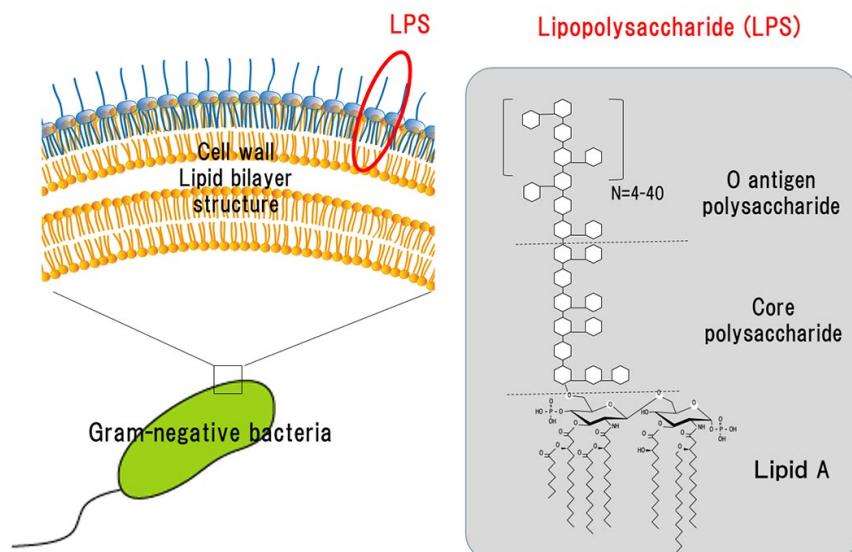


Figure 1.3: Gram-negative bacteria and LPS

Lipoproteins (LPS) are molecules found in the outer membrane of Gram-negative bacteria, which are a type of bacteria characterized by their cell wall structure. LPS is an endotoxin, meaning it's released when Gram-negative bacteria die and their cell walls break down. LPS plays a significant role in the pathogenesis of bacterial infections and is known to trigger an immune response in the host organism. When it enters the bloodstream it can activate immune cells such as macrophages and stimulate the release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha.

In the gut, Gram-negative bacteria, such as *Escherichia coli* and *Bacteroides* species, can release LPS as part of their normal life cycle, however the majority of LPS produced by these bacteria is usually contained within the bacteria cell wall and does not enter the bloodstream or cause systemic effects under normal circumstances. The disruption of the gut barrier, which occurs in conditions like intestinal inflammation, increased intestinal permeability or dysbiosis, can lead to increased translocation of bacteria and

their products, including LPS, from the gut lumen into the bloodstream. This can result in systemic exposure to LPS and activation of immune responses, inflammation, and potential health problems.

1.3.3 Bifidobacterium

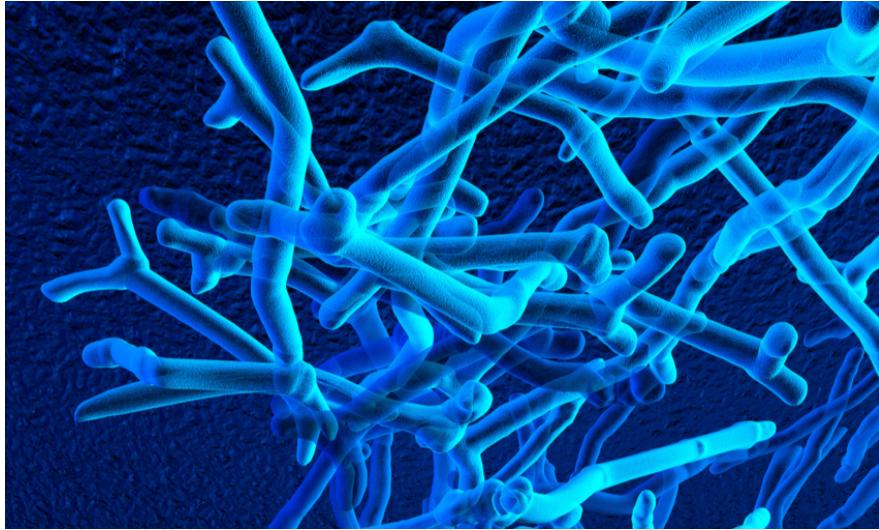


Figure 1.4: Bifidobacteria

Bifidobacteria, a genus of beneficial bacteria commonly found in the gut microbiota, have been shown to have several mechanisms through which they can counteract the effects of LPS.

1. **Reduction of LPS-producing bacteria:** They can help maintaining a healthy balance of gut bacteria by competing with and inhibiting the growth of LPS-producing bacteria, particularly Gram-negative bacteria like Escherichia coli. By reducing the abundance of LPS-producing bacteria, Bifidobacterium can help decrease LPS levels in the gut.
2. **Improvement of gut barrier function:** Bifidobacteria have been shown to enhance intestinal barrier function by promoting the integrity of the gut epithelium. By strengthening the gut barrier, Bifidobacteria can help prevent the translocation of LPS from the gut lumen into the bloodstream, reducing systemic exposure to LPS and its inflammatory effects.
3. **Modulating of immune responses:** Bifidobacteria can modulate immune responses in the gut, including reducing inflammation and cytokine production in response to LPS stimulation. Bifidobacteria can promote a balanced immune response preventing excessive inflammation and immune activation triggered by LPS.
4. **Binding and neutralization of LPS:** Some strains of Bifidobacteria have been shown to bind to LPS molecules, effectively neutralizing their pro-inflammatory effects. By sequestering LPS in the gut lumen, Bifidobacterium can prevent LPS from interacting with immune cells and inducing inflammation

1.4 Vagus Nerve

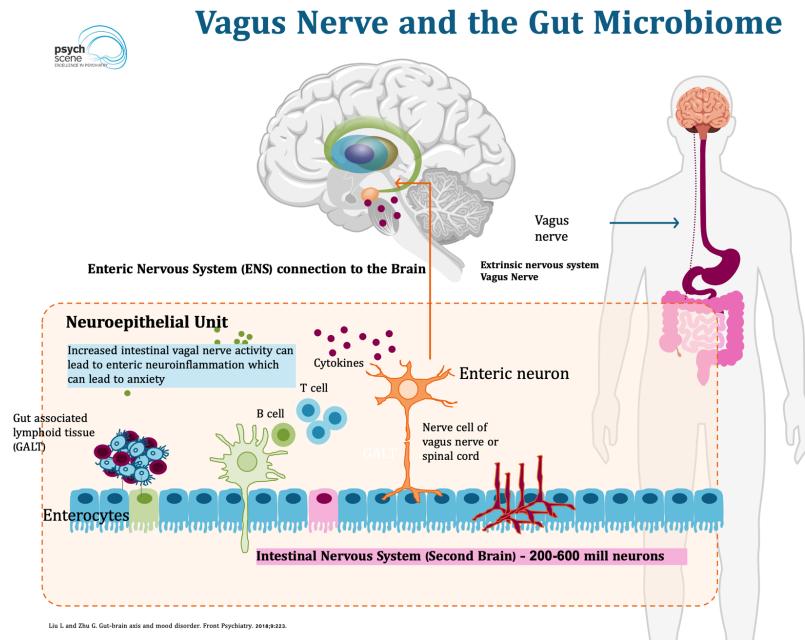


Figure 1.5: Gut brain axis

The vagus nerve, also known as the tenth cranial nerve, is a major nerve in the body that originates in the brainstem and extends through the neck, thorax, and abdomen. It is part of the parasympathetic nervous system, which regulates many involuntary functions.

This nerve has both sensory and motor functions. Sensory fibers of the vagus nerve transmit information from various organs to the brain, while motor fibers carry signals from the brain to these organs, influencing their activity.

Functions of the vagus nerve include regulating heart rate by affecting the heart's pacemaker, controlling digestion by influencing gastric secretion and motility, and modulating breathing patterns. It also plays a role in promoting relaxation and restorative processes in the body.

Additionally, the vagus nerve contributes to the innervation of organs such as the larynx, pharynx, esophagus, heart, lungs, stomach, and intestines. It is involved in modulating inflammation, immune responses, and mood, highlighting its widespread influence on bodily functions.

Due to its significance, the vagus nerve has been a subject of medical research, with interventions like vagus nerve stimulation explored as potential treatments for various conditions including epilepsy, depression, and inflammatory disorders.

2. System Description

The goal of our work is to describe the process of two independent systems, the gut and the Central Nervous System (CNS), and the possible relation that binds the gut dysbiosis with the Parkinson's Disease (PD). In particular our focus is on the aggregation process of the α -synuclein that ends with the formation of agglomerates called Lewy bodies.

As discussed before, the two systems are independent so the CNS presents a network of neurons and the α -synuclein inside the environment. For the gut we decided to represent the Gram-negative cells and the Bifidobacterium. Both systems start first from a state of equilibrium, then according to a certain level of probability they can move to a state of disease. Regarding the CNS we can have the aggregation of misfolding proteins that can lead to the formation of Lewy bodies. The gut may be subject to dysbiosis where the rate of creation of LPS exceeds the rate of cleaning of the Bifidobacterium. Gut dysbiosis increases the production of α -synuclein in the environment. This α -synuclein can reach the CNS through the vagus nerve and generate a overpopulation of α -synuclein.

2.1 Model used

As written before we individuated two environments: the CNS and the gut. The CNS is composed by the neurons and the α -synuclein proteins. The gut is composed by the Bifidobacterium, the Gram-negative bacteria and the LPS.

About the α -synuclein we did not find specific behaviour in both CNS and gut, we therefore have the same behavior in both the systems. To simplify our model, we considered an α -synuclein agent as a set of multiple α -synuclein molecules.

2.1.1 CNS Environment

The environment (the CNS) is defined as the tuple:

$$\langle E, S_0, \tau \rangle$$

Where E is the set of states, it contains the following elements:

- S_0 : Balanced state where the proteins fold normally. It's the initial state
- S_1 : State where we can find some aggregation
- S_2 : State onset of the disease
- S_3 : State of the spread of the disease

- S_4 : State of death

τ is the list of runs and contains the following:

- $r_1: S_0 \xrightarrow{\text{folding}} S_1$
- $r_2: S_0 \xrightarrow{\text{presenceAggregation}} S_1$
- $r_3: S_0 \xrightarrow{\text{presenceAggregation}} S_1 \xrightarrow{\text{presenceOligomers}} S_2$
- $r_4: S_0 \xrightarrow{\text{presenceAggregation}} S_1 \xrightarrow{\text{presenceOligomers}} S_2 \xrightarrow{\text{spreadProteins}} S_3$
- $r_5: S_0 \xrightarrow{\text{presenceAggregation}} S_1 \xrightarrow{\text{presenceOligomers}} S_2 \xrightarrow{\text{spreadProteins}} S_3 \xrightarrow{\text{dead}} S_4$
- $r_6: S_0 \xrightarrow{\text{presenceAggregation}} S_1 \xrightarrow{\text{clearing}} S_0$
- $r_7: S_0 \xrightarrow{\text{presenceAggregation}} S_1 \xrightarrow{\text{presenceOligomers}} S_2 \xrightarrow{\text{semicleared}} S_1$
- $r_8: S_0 \xrightarrow{\text{presenceAggregation}} S_1 \xrightarrow{\text{presenceOligomers}} S_2 \xrightarrow{\text{clearing}} S_0$

2.1.2 α -synuclein

The α -synuclein is defined as an reactive agent and is composed by the tuple

$$\langle E, Per, Ac, see, do, action \rangle$$

The sets E , Per and Ac represent the states of the environment, the perceptions of the agent and its actions respectively and are defined as follows:

- $E = \{ S_0, S_1, S_2, S_3, S_4 \}$
- $Per = \{ \text{nearNeurons} \}$
- $Ac = \{ \text{binding} \}$

$$\begin{aligned} see &: S_0 \rightarrow \text{nearNeuronssyn} \\ action &: \text{nearNeurons} \rightarrow \text{binding} \\ do &: \text{binding} \times S_0 \rightarrow S_1 \end{aligned}$$

2.1.3 Neuron

The Neuron is defined as an adaptive agent and is composed by the tuple

$$\langle I, E, Per, Ac, folded, see, next, do, action \rangle$$

where the set I is the set of internal states and contains the following items:

- $folded$: balanced state of the neuron
- $misfolded$: the neuron contains misfolded α -synuclein
- $oligomers$: the neuron contains misfolded proteins aggregated with each other

- *LewyBodies*: the neuron contains Lewy bodies

The sets E , Per and Ac represent the states of the environment, the perceptions of the agent and its actions respectively and are defined as follows:

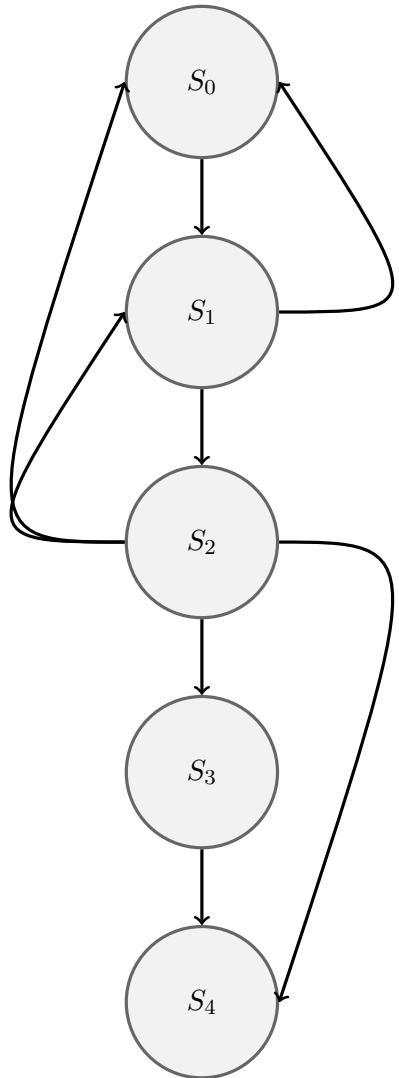
- $E = \{ S_0, S_1, S_2, S_3, S_4 \}$
- $Per = \{ InsideVariable \}$
- $Ac = \{ folding, misfolding, aggregate, spread, clean \}$

see : $S_0 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow folding$
do : $folding \times S_0 \rightarrow S_0$
next : $folded \times InsideVariable \rightarrow folded$

see : $S_0 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow misfolding$
do : $misfolding \times S_0 \rightarrow S_1$
next : $folded \times InsideVariable \rightarrow misfolded$

see : $S_1 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow aggregate$
do : $aggregate \times S_1 \rightarrow S_2$
next : $misfolded \times InsideVariable \rightarrow oligomers$
see : $S_2 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow spread$
do : $spread \times S_2 \rightarrow S_3$
next : $aggregate \times InsideVariable \rightarrow aggregate$
see : $S_3 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow aggregate$
do : $aggregate \times S_3 \rightarrow S_4$
next : $aggregate \times InsideVariable \rightarrow Lewybodies$
see : $S_2 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow aggregate$
do : $aggregate \times S_2 \rightarrow S_4$
next : $aggregate \times InsideVariable \rightarrow Lewybodies$
see : $S_1 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow clean$
do : $clean \times S_1 \rightarrow S_0$
next : $misfolded \times InsideVariable \rightarrow folded$
see : $S_2 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow clean$
do : $clean \times S_2 \rightarrow S_1$
next : $aggregate \times InsideVariable \rightarrow misfolded$
see : $S_2 \rightarrow InsideVariable$
action : $InsideVariable \rightarrow clean$
do : $clean \times S_2 \rightarrow S_0$
next : $aggregate \times InsideVariable \rightarrow folded$

2.1.4 CNS Automata used



2.2 Gut Environment

$$\langle E, S_0, \tau \rangle$$

Where E is the set of states and contains the following elements:

- S_0 : Balanced state
- S_1 : State onset of the disease
- S_3 : Dysbiosis
- S_4 : Spread α -synuclein

τ is the list of runs and contains the following:

- $r_1: S_0 \xrightarrow{\text{cleaning}}$

- $r_2: S_0 \xrightarrow{\text{malfunction}} S_1$
- $r_3: S_0 \xrightarrow{\text{malfunction}} S_1 \xrightarrow{\text{unbalancedLPSlevel}} S_2$
- $r_4: S_0 \xrightarrow{\text{malfunction}} S_1 \xrightarrow{\text{cleaning}} S_0$
- $r_5: S_0 \xrightarrow{\text{malfunction}} S_1 \xrightarrow{\text{unbalancedLPSlevel}} S_2 \xrightarrow{\text{spreadAlpha}} S_3$

2.2.1 Gram-negative cells

The Gram-negative cells are adaptive agents defined by:

$$\langle I, E, Per, Ac, balanced, see, next, do, action \rangle$$

where I is the set of internal states and contains the following items:

- *balanced*, balanced state of the cell
- *production*, production of LPS inside the gut
- *dead*, dead of the agent, release of many LPS

The sets E , Per and Ac represent the states of the environment, the perceptions of the agent and its actions respectively and are defined as follows:

- $E = \{ S_0, S_1, S_2 \}$
- $Per = \{ globalVariable \}$
- $Ac = \{ production, release \}$

The rest of the elements are the functions of the agent, here are their possible values.

$see : S_0 \rightarrow globalVariable$
 $action : globalVariable \rightarrow production$
 $do : production \times S_0 \rightarrow S_0$
 $next : balanced \times globalVariable \rightarrow balanced$

$see : S_0 \rightarrow globalVariable$
 $action : globalVariable \rightarrow production$
 $do : production \times S_0 \rightarrow S_1$
 $next : production \times globalVariable \rightarrow balanced$

$see : S_1 \rightarrow globalVariable$
 $action : globalVariable \rightarrow production$
 $do : production \times S_1 \rightarrow S_2$
 $next : production \times globalVariable \rightarrow balanced$

$see : S_2 \rightarrow globalVariable$
 $action : globalVariable \rightarrow release$
 $do : release \times S_2 \rightarrow S_3$
 $next : release \times globalVariable \rightarrow dead$

2.2.2 LPS

LPS is a reactive agent used also as a threshold for the system.

- Per = { presenceBifidoAgent }
- Ac = { removeItself }

see : $S_i \rightarrow presenceBifidoAgent$
action : $presenceBifidoAgent \rightarrow removeItself$
do : $removeItself \times S_i \rightarrow S_i$
With i from 0 to 4

see : $S_i \rightarrow presenceBifidoAgent$
action : $presenceBifidoAgent \rightarrow removeItself$
do : $removeItself \times S_i \rightarrow S_i + 1$
With i from 0 to 3

2.2.3 Bifidobacterium

The Bifidobacterium is a reactive agent identified by:

- Per = { presenceLPS }
- Ac = { clearing, malfunction, unbalancedLPSLevel }

see : $S_0 \rightarrow presenceLPS$
action : $presenceLPS \rightarrow malfunction$
do : $malfunction \times S_0 \rightarrow S_1$

see : $S_1 \rightarrow presenceLPS$
action : $presenceLPS \rightarrow unbalancedLPSlevel$
do : $unbalancedLPSlevel \times S_1 \rightarrow S_2$

see : $S_1 \rightarrow presenceLPS$
action : $presenceLPS \rightarrow clearing$
do : $clearing \times S_1 \rightarrow S_2$

2.2.4 Gut Neuron

It's defined as reactive agent. Its role is to send the α -synuclein from the gut to the CNS.

- Per = { presenceAlpha }
- Ac = { spreadAlpha }

see : $S_2 \rightarrow presenceAlpha$
action : $presenceAlpha \rightarrow spreadAlpha$
do : $spreadAlpha \times S_2 \rightarrow S_3$

2.2.5 α -synuclein

The α -synuclein is defined as a reactive agent and is composed by the tuple

$$\langle E, Per, Ac, see, do, action \rangle$$

The sets E , Per and Ac represent the states of the environment, the perceptions of the agent and its actions respectively and are defined as follows:

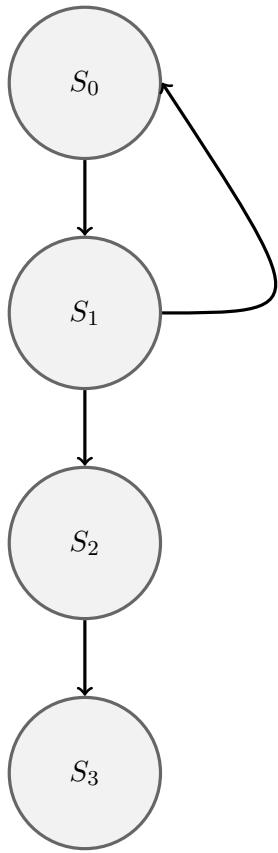
- $E = \{ S_0, S_1, S_2, S_3 \}$
- $Per = \{ nearNeurons \}$
- $Ac = \{ binding \}$

$see : S_2 \rightarrow nearNeuronssyn$

$action : nearNeurons \rightarrow Binding$

$do : Binding \times S_2 \rightarrow S_3$

2.2.6 GUT Automata used



2.2.7 Transition function

The transition function is the function that permits the movement of an α -synuclein agent from the Gut environment to the CNS environment.

$$T = \{ S_{gut}, S_{CNS}, \alpha\text{-synuclein, gutneuronagent} \}$$

- S_{gut} : the state of the Gut
- $S_{CNS(i)}$: a subset of the CNS state composed by $\{S_1, S_2, S_3\}$

$$T : S_{gut} \times S_{CNS(i)} \times \alpha\text{-synuclein} \times \text{gutneuronagent} \rightarrow S_{CNS(i)}$$

$$T : S_{gut} \times S_{CNS(i)} \times \alpha\text{-synuclein} \times \text{gutneuronagent} \rightarrow S_{CNS(i+1)}$$

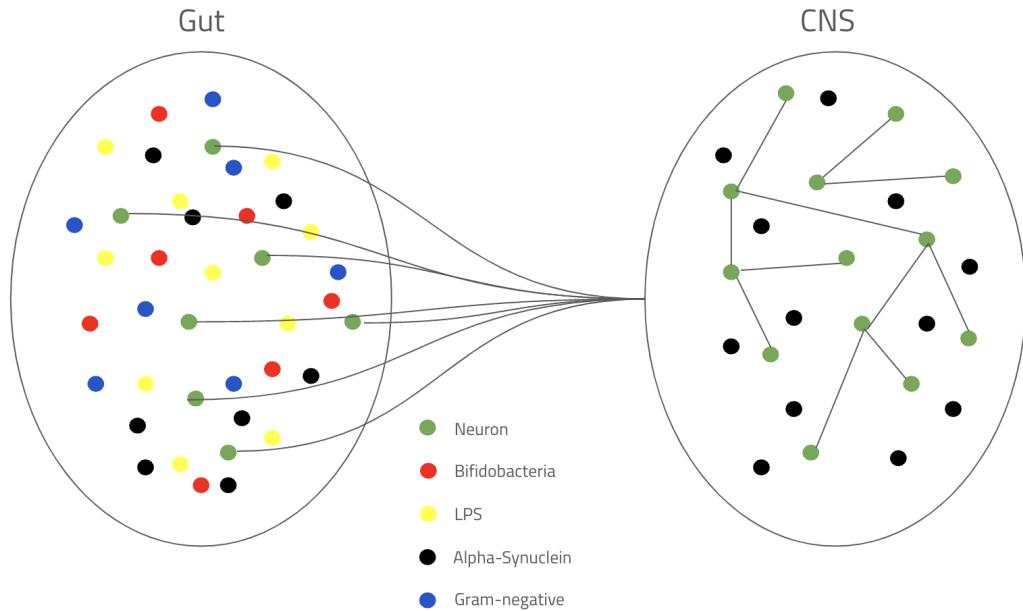


Figure 2.1: Our representation of the two systems

3. Model implementation

To represent the model we used the Python library **repast4py**. It is a open source agent-based modeling and simulation software, built on Repast HPC, that provides the ability to build large, distributed agent-based models that span multiple processing cores. Since we have two independent environments we first describe the two systems and then we describe the interaction between them. As we said before the goal of this project is to show how gut dysbiosis can lead to Parkinson's disease through the misfolding of the α -synuclein that starts in the gut and reaches the CNS.

3.1 CNS implementation

Based on the formal model we have 2 agents in the CNS: the alpha-synuclein and the Neuron. To represent the CNS we use a single context with two different projections on it.

3.1.1 Neuron

The neuron is represented as a single entity with some internal values. These values represent the level of α -synuclein, the level of misfolding proteins, the oligomers and the Lewy bodies inside the neuron. During the simulation, every time the neuron runs the `step()` method it increases the level of proteins inside itself. The `step()` method is also used to control the level of misfolding proteins, oligomers and Lewy bodies. Based on some probability this method can simulate the behaviour inside a neuron cell:

- Increase production of α -synuclein
- Misfolding of the α -synuclein
- Aggregation of the proteins in oligomers
- Aggregation of the oligomers in Lewy bodies
- Removal of misfolded proteins by the cleaning mechanisms

Every point in the previous list has a specific value of probability, this can change based on the level of proteins inside the cell, so the higher is the level of the proteins in the cell the higher is the probability to have misfolding or aggregation in oligomers or Lewy bodies. The level of the misfolding proteins is also used to set a threshold for the exchange of proteins with other neurons. When the misfolding level reaches a fixed amount the neuron transfers a number of proteins to a neighbouring neuron. So it decreases the level of the proteins inside itself to increase the level of another neuron.

```
random = rng.uniform()
# Alpha-syn is increased when there are no Lewy bodies
# yet
if self.lewy_bodies_level < 50:
    self.alpha_synuclein_level += rng.integers(1, 500)
# Chance of alpha-syn reduction
if random < 0.5 and self.alpha_synuclein_level > 1000:
    self.alpha_synuclein_level -= rng.integers(1, 1000)
# The alpha-syn misfolds with a lower probability,
# which increases with its concentration.
# Misfolding increases the misfolding level and
# decreases normal alpha-syn
if (self.alpha_synuclein_level > 1000 and random < 0.3
    + self.alpha_synuclein_level / 100000
    + self.oligomer_level / 1000
    + self.lewy_bodies_level / 100
    + params['misfolding_pb'] / 1000):
    add_remove = rng.integers(1, 250)
    self.misfolding_level += add_remove
    self.alpha_synuclein_level -= add_remove

# Formation of alpha-syn oligomers, probability is
# increased based on the levels
if (self.misfolding_level > 1000 and
    rng.uniform() < 0.05 + self.misfolding_level / 1000
    + self.oligomer_level / 1000
    + self.lewy_bodies_level / 100
    + params['oligomers_pb']/1000):
    variation = rng.integers(0, 50)
    self.misfolding_level -= variation * 10
    self.oligomer_level += variation

# Formation of Lewy bodies, probability is increased
# based on the levels
if (self.oligomer_level > 300 and
    rng.uniform() < 0.02 + self.oligomer_level / 1000
    + self.lewy_bodies_level / 1000
    + params['lewy_bodies_pb'] / 1000):
    variation = rng.integers(0, 5)
    self.oligomer_level -= variation * 10
    self.lewy_bodies_level += variation

# Neuron internal mechanisms try to reduce the amount
# of misfolded proteins
if self.misfolding_level > 100:
    self.misfolding_level -= rng.integers(50, 100)
if self.oligomer_level > 5:
    self.oligomer_level -= rng.integers(1, 5)
```

```

if self.misfolding_level < 500 and model.contains(self):
    model.neuron_spreaders.remove(self)
    self.received_misfolding = False
    return -1
if self.misfolding_level > 500 and not model.contains(self):
    model.neuron_spreaders.append(self)
    return 1
if self.misfolding_level > 500:
    self.state = 1
    if self.oligomer_level > 150:
        self.state = 2
        if self.lewy_bodies_level > 50:
            self.state = 3
return 0

```

3.1.2 α -synuclein

The **Alpha** agent represents a set of α -synuclein. Since it is a set of proteins it contains values that represent the folding and misfolding proteins inside of the object. It is a mobile agent that can interact with the neuron agent. When it reaches the position of a neuron the proteins fuses with the neuron cell. During this process the levels of the proteins and misfolding inside the **Alpha** agent are used to increase their respective levels within the neuron.

```

def step(self):
    grid = model.grid if self.in_CNS else model.gut_grid

    xy_dirs = rng.choice(Alpha.RNDOFFSETS, size=2)
    if self.in_CNS:
        model.move(self, self.pt.x + xy_dirs[0],
                   self.pt.y + xy_dirs[1])
    else:
        model.move_gut(self, self.pt.x + xy_dirs[0],
                       self.pt.y + xy_dirs[1])
    self.pt = grid.get_location(self)
    nghs = model.ngh_finder.find(self.pt.x, self.pt.y)
    at = DiscretePoint(0, 0)
    for ngh in nghs:
        at._reset_from_array(ngh)
        for agent in grid.get_agents(at):
            if self != agent and agent.uid[1] == Neuron.TYPE:
                agent.misfolding_level += self.misfolding_level
                agent.alpha_synuclein_level +=
                    self.alpha_synuclein_level
                self.fusion = True

    if self.energy < 0:
        self.is_alive = False
    if model.runner.schedule.tick % params['tick_life_alpha']:
        if self.energy > 0:

```

```
self.energy == rng.uniform()
```

3.1.3 CNS network

We have a network projection to represent the connection between the neurons inside the CNS. This network is used to represent the exchange of the proteins inside the CNS. Instead to represent the physical interaction between the agents we used a grid projection where both α -synuclein and neuron are added. Thanks to the Moore grid we are able to speed up the computation of the neighbours for the α -synuclein objects in the grid.

3.2 GUT implementation

Based on the model, the gut contains 5 different agents:

1. Gram-negative cell
2. LPS
3. Gut Neuron
4. Bifidobacteria
5. α -synuclein

We decided to use only one projection to represent the Gut. This because we want to represent only the physical interaction between the agent inside the space.

3.2.1 Gram-negative cell

The Gram-negative cells are mobile objects that for each tick of the simulation can move inside the space. Their main function is to generate LPS agents during their movement based on a flag inside of the object. This flag can change based on some probability level, which is not a fixed value but can change based on time or the inflammation level of the gut. When the flag changes its value to true, the cell generates a single LPS object and then reset it flag to false. The Gram-negative agents can also release a large amount of LPS inside the gut. This also happens according to a probability level, as the probability before it is under the influence of time and inflammation level of the gut, after the release the agent is removed from the system. This type of object interacts only with bifidobacteria agents, this interaction triggers an action done by the bifidobacteria.

```
def step(self):
    xy_dirs = rng.choice(GramNegative.RNDOFFSETS, size=2)
    model.move_gut(self, self.pt.x + xy_dirs[0], self.pt.y +
    xy_dirs[1])
    self.pt = model.gut_grid.get_location(self)
    if rng.uniform() < 0.06 + model.lps_count / 100000 +
        params['dysboisi_probability'] / 100 +
        model.runner.schedule.tick / 10000:
        self.generateLPS = True
```

```

death_pb = rng.uniform()
if death_pb < 0.01:
    self.remove = True
    if (death_pb < 0.0001
        + params['release-probability'] / 5000):
        self.release = True

```

3.2.2 LPS

These objects are used to determine the level of dysbiosis or inflammation of the gut. They are generated from Gram-negative cell. They interact only with Bifidobacteria. When this interaction happens the LPS are removed from the system. They are mobile agents so they can move inside the space of the gut.

```

def step(self):
    xy_dirs = rng.choice(LPS.RNDOFFSETS, size=2)
    model.move_gut(self, self.pt.x + xy_dirs[0], self.pt.y
                   + xy_dirs[1])
    self.pt = model.gut_grid.get_location(self)

```

3.2.3 Bifidobacteria

Their main role is to kill the malicious cells, (Gram-negative, LPS).

For each tick of the simulation they move inside the space, when they meet a Gram-negative or LPS object they remove it from the space. Based on a threshold, when the agent removes an object it may remove also itself. Bifidobacteria are generated every fixed amount of ticks.

```

def step(self):
    grid = model.gut_grid
    xy_dirs = rng.choice(LPS.RNDOFFSETS, size=2)
    model.move_gut(self, self.pt.x + xy_dirs[0], self.pt.y +
                   xy_dirs[1])
    self.pt = grid.get_location(self)
    nghs = model.ngh_finder.find(self.pt.x, self.pt.y)
    at = DiscretePoint(0, 0, 0)
    for ngh in nghs:
        at._reset_from_array(ngh)
        for obj in grid.get_agents(at):
            if self != obj and (obj.uid[1] == LPS.TYPE or
                                obj.uid[1] == GramNegative.TYPE):
                obj.remove = True
    if rng.uniform() < params['pr_dead_bifido']:
        self.remove = True

```

3.2.4 Gut neuron

It is a static agent and there is a fixed amount of it inside of the gut. For each tick it checks if in the neighbour cells there are some α -synuclein agents. If it finds them it

changes a flag in the agent. The flag is used to in the next tick to move the agent from the Gut to the CNS.

```
def step(self):
    nghs = model.ngh_finder.find(self.pt.x, self.pt.y)
    at = DiscretePoint(0, 0, 0)
    for ngh in nghs:
        at._reset_from_array(ngh)
        for obj in model.gut_grid.get_agents(at):
            if self != obj and obj.uid[1] == Alpha.TYPE:
                obj.spread = True
```

3.2.5 α -synuclein

It is a mobile agent. It represents a set of proteins so in the object there are value that represent the level of folding proteins and misfolding proteins inside the set. They are generated based on the level of LPS inside the Gut. More LPS are present more proteins are generated.

3.2.6 Projection used

We decide to represent the gut as a grid. For every tick of the simulation the agent can do a step of [-1,+1] in the X,Y axis. To check the presence of the neighbours on the various agent we used the Moore Grid to speed up the computation.

3.2.7 Vagus nerve implementation

In theory the movement of the proteins from the Gut to the CNS is done through the vagus nerve. We decided to not represent the vagus nerve because according with the goal of the project we want to represent the gut dysbiosis that leads to PD through the α -synuclein misfolding. An implementation of the vagus nerve would not have changed anything for the simulation. Since the vagus nerve represents only a communication channel from a point A to a point B we have decided to simplify this step by removing from the implementation the representation of the vagus nerve, by doing that we saved computational cost. In our implementation the vagus nerve is represented by the Gut neuron that simulate a connection between the Enteric Nervous System and the Central Nervous System. When a protein hits a Gut neuron instead of enter in the vagus nerve and move from the Gut to the Brain. Protein is simply removed from the intestine and put into the brain. Because the main goal of our implementation it is not to represent the movement of the protein.

3.3 Implementation parameters

```
random.seed: 42
stop.at: 1000
alpha.count: 400
world.width: 200
world.height: 200
counts_file: './output/agent_counts.csv'
```

```

agent_pos_gut: './output/agent_pos_gut.csv'
agent_pos_cns: './output/agent_pos_cns.csv'
file_cns_network: 'CNSNetwork'
agent_features: './output/agent_features.csv'
misfolding_pb: 0
oligomers_pb: 0
lewy_bodies_pb: 0
dysboisi_probability: 10
release_probability: 5
flag_enabling_move_between_system: True
attiva_CNS: True
attiva_gut: True
gut_neuron: 20
gram_negative: 10
bifidobacteria: 10
pr_dead_bifido: 0.008
lps_release: 10
folding_in_gut: 2000
oligomers_in_gut: 100
generation_alpha_gut: 1000
tick_life_alpha: 10
starting_folding_level: 1000
starting_oligomer_level: 10
alpha_generation_CNS: 100
folding_level_generation_alpha: 2000
misfolding_level_generation_alpha: 10

```

- misfolding_pb: probability of misfolding inside neurons
- oligomers_pb: probability that misfolded proteins aggregate into oligomer inside neurons
- lewy_bodies_pb: probability to go from oligomers to Lewy bodies inside neurons
- dysbiosis_probability: probability of gram cell to create one LPS
- release_probability: probability of gram cell to release LPS
- flag_enabling_move_between_system: flag that enables the moving of α -synuclein from the Gut to the CNS
- attiva_CNS: flag to enable the step method of the CNS
- attiva_gut: flag to enable the step method of the Gut
- gut_neuron: number of neurons inside the Gut
- gram_negative: number of gram negatives generated at the start and at every tick
- bifidobacteria: number of bifidobacteria generated at the start and at every tick
- pr_dead_bifido: probability of bifidobacteria to die
- lps_release: number of LPS agents released by the gram-negative

- folding_in_gut: level of folding proteins inside an α -synuclein in the Gut
- oligomer_in_gut: level of oligomers inside an α -synuclein agent in the Gut
- generation_alpha_gut: amount of α -synuclein generated in the gut. This number is used to divide the LPS number in the Gut.
- tick_life_alpha: number of ticks to decrement the α -synuclein life
- starting_folding_level: starting folding level of α -synuclein agent generated in the Gut
- starting_oligomer_level: starting level of oligomers of α -synuclein agents generated in the Gut
- alpha_generation_CNS: number of ticks to generate α -synuclein
- folding_level_generation_alpha: folding level of proteins inside an α -synuclein agent generated each amount of tick in CNS
- misfolding_level_generation_alpha: misfolding level of proteins inside an α -synuclein agent generated each amount of tick in CNS

4. Simulation

To test our model, we performed three different simulations to evaluate how our model performs in different conditions.

4.1 Simulation in normal conditions

In the first simulation we tested the system under normal conditions. So we set the global parameters in a state where we can not bring the system on an illness state. The graph below represents the number of agents inside the Gut and inside the CNS. We can see based on the behaviour that we chose that the system maintains a balanced state. The main thing that we can notice is that in the end of the simulation we have a peak of malicious agents. This peak depends on the fact that our simulation is under the influence of time. The more the simulation goes on, the higher is the probability to have inflammation in the gut.

The same thing happened in the CNS, where we find that half the neurons contain misfolding proteins. As the Gut system, the CNS is also under the influence of time.

Another important thing to highlight is the number of agents in the simulation. There are approximately 3,600, plus the neurons in the Gut and the neurons in the CNS. This number is not so large to cause performance problems in the simulation but if we set a larger number of ticks things could change in that regard, since the way the number of agent grows is similar to an exponential function.

4.2 Simulation of weak Gut

In this case we take as example a patient that has some genetic predispositions. In the simulation we increase the level of inflammation probability by increasing the level of dysbiosis probability and release probability in the gut. These parameters are linked to the amount of LPS and Gram-negative cells. In this case we increment the probability of a Gram-negative to generate a LPS or the probability to release an amount of LPS.

Similar to the previous graph we have an exponential trend of the curves representing the inflammatory agents. The level of Gram-negative remains balanced for the entire simulation because increasing the probability of the release means to increase the probability to be destroyed. In this type of simulation the impact that time has on the simulation is much less evident. This is because since the LPS are generated with more frequency it means that the threshold for which the LPS are generated becomes increasingly lower as the simulation progresses. More LPS are present in the simulation, more is the probability have LPS generated from Gram-negative. We can see from the graph that we have much more agents than before within the simulation, now only the LPS are more than 10.000. The CNS graph shows us that the movement of the α -synuclein

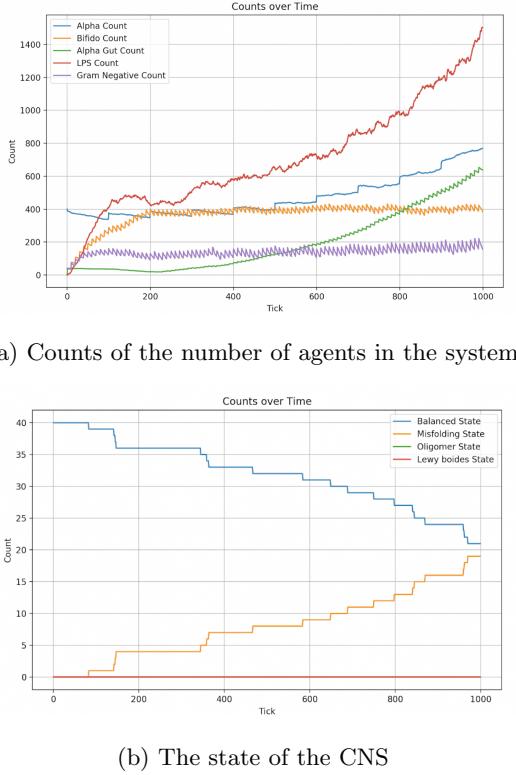


Figure 4.1: Graphs showing how the system remains in a state of equilibrium

from the gut to the brain has as result that Lewy bodies start forming in some neurons.

4.3 Simulation of the onset of the disease in CNS

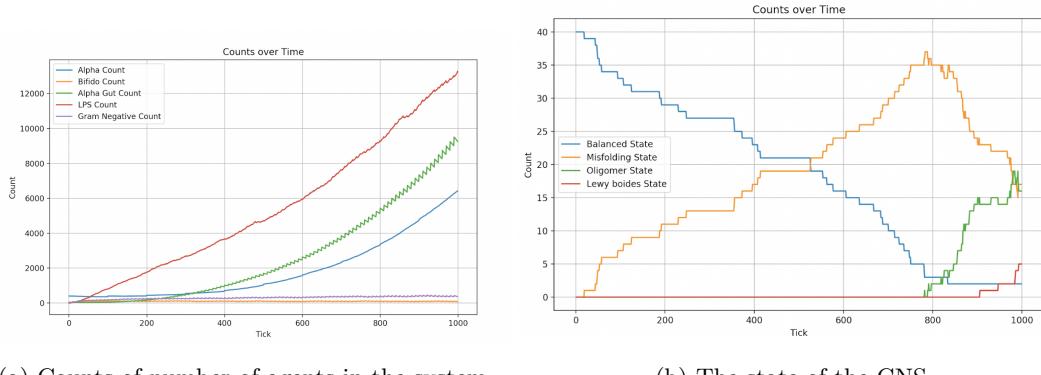
In the third simulation we took as example a patient with genetic predisposition for Parkinson's disease. In this simulation we changed all the parameters that involve the probability to get the Parkinson's disease directly in the CNS.

As we can see in the graph we have a balanced number of agents inside the gut, but we have a constant increment of α -synuclein agents in the brain. In the other graph that monitors the state of the neurons in the CNS we notice that we have a lot of dead neurons because of the presence of Lewy bodies, and also numerous neurons that contain oligomers.

4.4 Considerations on the three simulations

The purpose of these three simulations is to show how the two independent systems can have different behaviours based on the interaction. In the first case since we are in a balanced state we have few interactions between the two systems. In this case since the gut and the CNS are in a balanced state we don't have the onset of the disease in neither of the two.

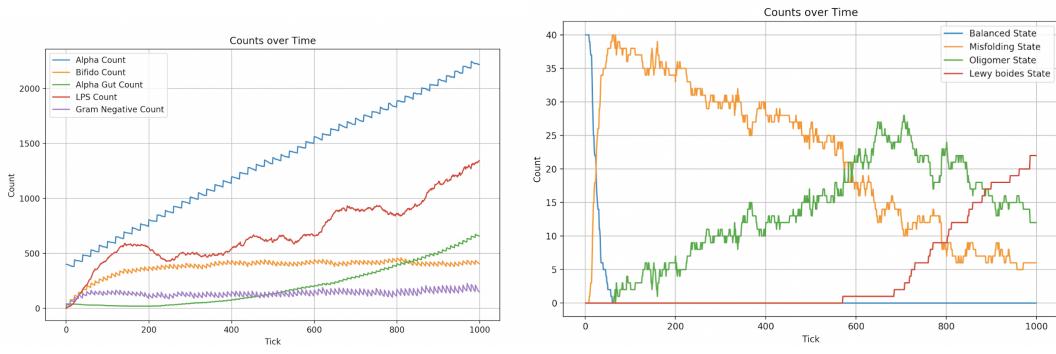
Things are different for the second simulation. Here the interactions between the two systems are important, in fact the disease starts first as inflammation in the Gut, after that more α -synuclein is exchanged between the Gut and the CNS. This type



(a) Counts of number of agents in the system

(b) The state of the CNS

Figure 4.2: Graphs showing the system with gut dysbiosis



(a) Counts the number of the agents in the system

(b) Represent the state of CNS

Figure 4.3: Graphs showing how Parkinson's starts from the brain

of exchange starts the spread of misfolded proteins in the brain, potentially leading to Parkinson's.

The third simulation it used to show how we can "disconnect" the CNS from the Gut. In this simulation we forced the onset of the disease directly into the brain. The proteins exchanged from the gut to the brain were a small amount. This system is completely independent from the Gut and it can get Parkinson's directly from the α -synuclein in the brain and inside the neurons.

4.5 Graphical interface

Regarding the user interface we decided to use the matplotlib library. It is a Python library mainly used for creating static, animated, and interactive visualizations in Python.

Our interface is a post simulation interface, this means that first we execute the simulation of our system and generate data for every tick, then we plot the data and create a graph and the simulation of the movement of the agents inside the environment. For the graph that shows the number of agent in the two systems we write in a CSV file number of the agents in the system at every tick. For this type of visual representation we used a graph where on the X-axis is the time and the Y-axis is the number of agents.

Different is the representation of the movement inside the environment. We used a CSV file, but in this file we plot for each tick the position of each agent in the simulation. So for example for tick 1 we have the position of a thousand agents. Once we obtain this huge file from the simulation we take a color code based on the id of the agent and their position, then we plot each agent as a point in a point graph. Since this is an animated graph for each tick we delete the previous points and create new ones for the following tick.

We have two different movement graphs, one for the Gut where we represent only the movement of the agents and one for the CNS. The movement graph for the CNS is a little bit different from that of the Gut. In the CNS we represented the movement of the α -synuclein inside the environment but also we decided to represent the state of the neurons based on the level of proteins inside the agent. To do that, in the CSV we saved the position of the agents and another variable that represents the state of the neurons. In the graph we can see how the color of the neurons change based on the level of the proteins inside them.

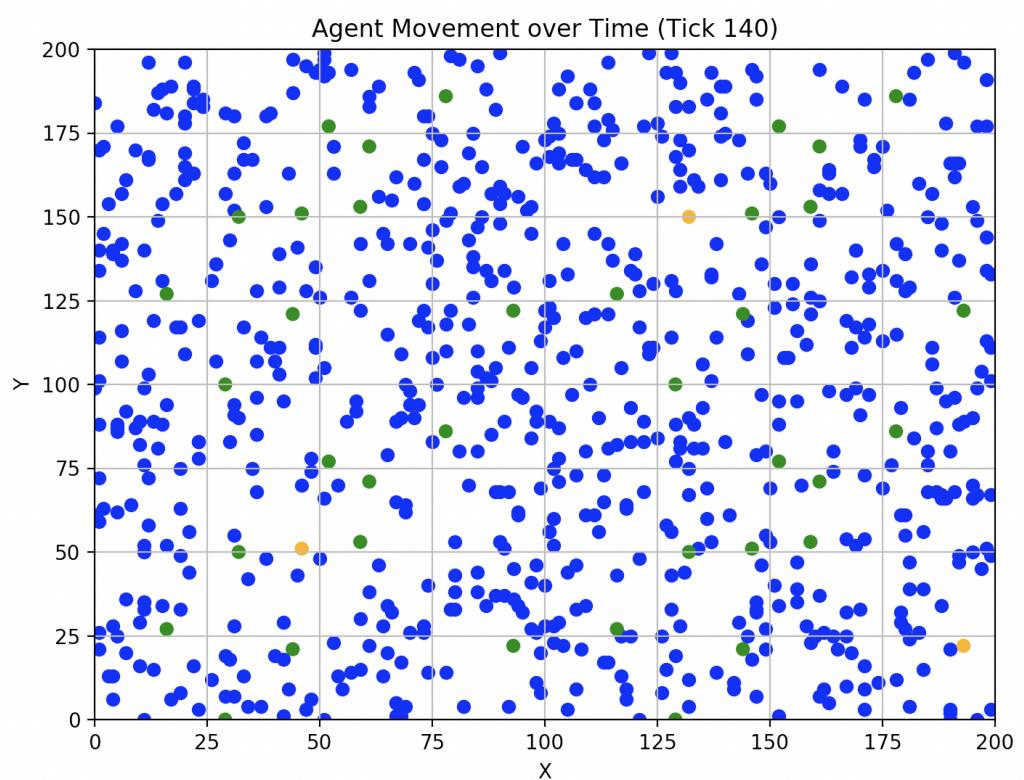


Figure 4.4: The movement of the agents inside the CNS

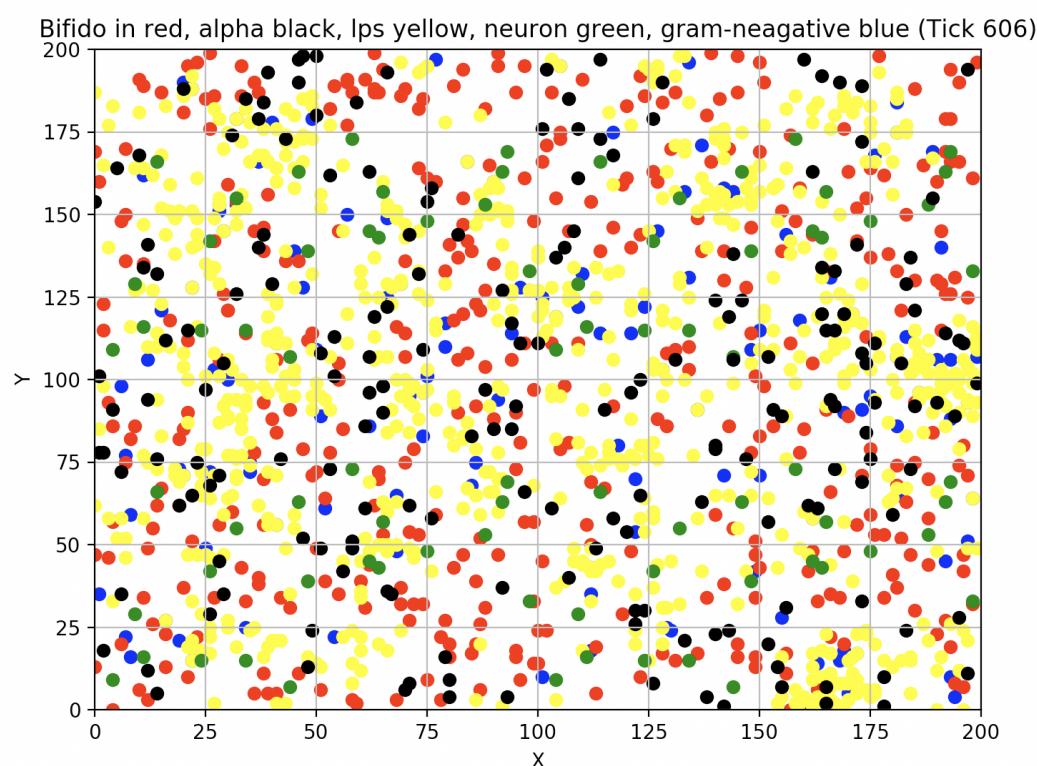


Figure 4.5: The movement of the agents inside the Gut

5. Conclusion

The goal of the project has been reached. We are able to demonstrate our thesis on how gut inflammation can lead to Parkinson's disease. It was a challenging project where we had to link the IT field and the Biological field, but fortunately we were able to use our previous work done for the Distributed Calculus and Coordination exam. We took the old project and revisited it for the CNS system and then realized an approximate version of the Gut system thanks to the research work done in the early stage of the project. Another challenge was using the *repast4py* framework, which was released very recently and thus had a very basic documentation. In conclusion we can consider ourselves satisfied with the work done.

5.1 Consideration

This project represents a simplification of the biological world that we analyzed. The main problem that we met is with the simulation execution. As we can see by the graph that counts the number of agents, the longer the simulation runs the greater the number of agents we need to manage and this caused performance problems. There are a few solutions that could solve this type of problem. The first one is the simplest and is to run the simulation on a much more powerful machine. Another solution is to change the way in which the agents are represented in the system. For example instead of having an agent that represents a cell, we can reformulate the model and have an agent that represents a group of cells. This type of solution can reduce a lot the number of agents in the simulation. We applied a similar strategy in our CNS system were the α -synuclein agent is actually a set of proteins and not just a single one. This type of solution requires a lot of work during the design phase because grouping more entities in a single one can lead to a less accurate simulation.