The Impact of the Gut Microbiota in Aged Rats on Spontaneous Seizure-like Discharges and Seizure Threshold: Mechanisms Involving Macrophages and Microglia

Abstract

The study investigates the impact of gut microbiota on spontaneous seizure-like discharges and seizure thresholds in aged rats, focusing on the mechanisms involving macrophages and microglia. The investigation employs a structured approach, encompassing animal models, gut microbiota analysis, seizure threshold measurement, and detailed analyses of macrophage and microglia activities.

Aging is associated with changes in gut microbiota composition, which may affect neurological function. This study uses aged rat models to explore the relationship between gut microbiota alterations and seizure activities. By analyzing gut microbiota profiles and correlating them with seizure data, the research aims to uncover potential microbial influences on seizure dynamics.

Methods include detailed procedural descriptions for analyzing gut microbiota, measuring seizure thresholds, and examining the roles of macrophages and microglia in the brain. Results indicate significant shifts in gut microbiota composition in aged rats, correlating with increased seizure-like discharges and altered seizure thresholds. Increased activity of macrophages and microglia suggests an inflammatory component contributing to seizure susceptibility.

The discussion interprets these findings, proposing mechanisms by which gut microbiota might influence brain inflammation and seizure activity through immune cell interactions. The study highlights the importance of gut-brain axis research in understanding age-related neurological disorders and suggests avenues for future research to explore therapeutic interventions targeting gut microbiota.

Introduction

The gut microbiota, a diverse and dynamic ecosystem of microorganisms residing in the gastrointestinal tract, has garnered significant attention for its profound impact on host health. This introduction sets the stage for exploring how age-related alterations in gut microbiota composition influence spontaneous seizure-like discharges and seizure thresholds in aged rats, with a focus on the mechanisms involving macrophages and microglia.

Gut Microbiota and Aging

As organisms age, the composition and diversity of their gut microbiota undergo substantial changes. These alterations often result in a decline in beneficial microbial species and a rise in pathogenic bacteria, leading to systemic inflammation and dysregulated immune responses. Such microbial imbalances, termed dysbiosis, are associated with various age-related diseases, including neurodegenerative disorders. The gut-brain axis, a bidirectional communication network connecting the gut microbiota and the central nervous system (CNS), plays a crucial role in this context. Through this axis, gut microbiota can influence brain function and behavior via the production of metabolites, neurotransmitters, and other signaling molecules.

Seizures and Neuroinflammation

Aging is commonly accompanied by an increased incidence of spontaneous seizure-like discharges and a lowered seizure threshold. Neuroinflammation, characterized by the activation of microglia and macrophages in the CNS, is a pivotal factor in the pathophysiology of seizures. Pro-inflammatory cytokines released by these immune cells can modulate neuronal excitability and

synaptic function, thereby heightening the susceptibility to seizures. Understanding the interaction between gut microbiota, neuroinflammation, and seizure activity is essential for developing novel therapeutic strategies.

Role of Macrophages and Microglia

Macrophages and microglia are the primary immune cells in the CNS, responsible for maintaining homeostasis and responding to injury or infection. In aged individuals, chronic activation of these cells can lead to sustained neuroinflammation. The gut microbiota can influence the activity of macrophages and microglia through modulation of systemic and CNS-specific immune responses, as well as direct effects of microbial metabolites. This study aims to elucidate the mechanisms by which gut microbiota alterations in aged rats affect the activity of these immune cells and contribute to seizure susceptibility.

Research Objectives

The primary objective of this study is to investigate the influence of gut microbiota on the occurrence of spontaneous seizure-like discharges and the seizure threshold in aged rats. Specifically, the study will:

1. **Characterize the Composition of Gut Microbiota**:
    - Utilize advanced sequencing techniques to analyze the gut microbiota composition in aged rats.
    - Compare microbial profiles between rats with and without seizure-like discharges.
2. **Assess Seizure Activity and Threshold**:
    - Monitor and record spontaneous seizure-like discharges using electroencephalography (EEG).
    - Determine the seizure threshold in response to specific pro-convulsant stimuli.
3. **Examine Macrophage and Microglia Activity**:
    - Analyze the activation state and distribution of macrophages and microglia in the brain tissue of aged rats.
    - Investigate the correlation between immune cell activity and seizure parameters.
4. **Explore Mechanistic Pathways**:
    - Identify potential signaling pathways linking gut microbiota composition to changes in seizure activity and threshold.
    - Examine the role of inflammatory mediators produced by macrophages and microglia in these pathways.

This comprehensive approach aims to provide insights into the intricate relationships between gut microbiota, aging, and neurological health, potentially uncovering novel therapeutic targets for age-related neurological disorders.

Background

The gut microbiota plays a pivotal role in maintaining overall health and homeostasis, influencing various physiological systems, including the central nervous system (CNS). In recent years, there has been growing interest in understanding how alterations in the gut microbiota composition, particularly in aged populations, may impact neurological functions and disorders. This section delves into the existing literature and foundational concepts that underpin the study of gut

microbiota in aged rats and its potential link to spontaneous seizure-like discharges and seizure thresholds, with a focus on the involvement of macrophages and microglia.

Gut Microbiota and Aging

Aging is associated with significant changes in the composition and diversity of the gut microbiota. These changes can lead to a decline in beneficial microbial species and an increase in pathogenic bacteria, contributing to systemic inflammation and altered immune responses. Studies have shown that aged individuals often exhibit dysbiosis—a microbial imbalance—which has been linked to various age-related diseases, including neurodegenerative disorders.

Gut-Brain Axis

The gut-brain axis is a bidirectional communication pathway between the gastrointestinal tract and the CNS. It involves complex interactions between the gut microbiota, immune system, and neural pathways. Through the production of metabolites, such as short-chain fatty acids (SCFAs), neurotransmitters, and other signaling molecules, the gut microbiota can influence brain function and behavior. In aged populations, disruptions in this axis may exacerbate neuroinflammatory conditions and contribute to neurological disorders.

Seizures and Neuroinflammation

Spontaneous seizure-like discharges and lowered seizure thresholds are often observed in aging animal models and humans. Neuroinflammation, characterized by the activation of microglia and macrophages in the CNS, is a key factor in the pathophysiology of seizures. Pro-inflammatory cytokines released by these immune cells can alter neuronal excitability and synaptic function, increasing the susceptibility to seizures.

Role of Macrophages and Microglia

Macrophages and microglia are the primary immune cells in the CNS, responsible for maintaining homeostasis and responding to injury or infection. In the context of aging and altered gut microbiota, these cells can become chronically activated, leading to sustained neuroinflammation. The mechanisms through which gut microbiota influence macrophage and microglia activity include modulation of systemic and CNS-specific immune responses, as well as direct effects of microbial metabolites on these cells.

Research Objectives

The primary objective of this study is to investigate how age-related changes in gut microbiota composition affect spontaneous seizure-like discharges and seizure thresholds in aged rats. Additionally, the study aims to elucidate the mechanisms involving macrophages and microglia that mediate these effects. Understanding these interactions could provide insights into novel therapeutic targets for managing age-related neurological disorders.

Summary

In summary, the background section sets the stage for exploring the intricate relationships between gut microbiota, aging, and neurological health. By examining the role of macrophages and microglia in mediating the effects of gut microbiota on seizure activity, this study aims to contribute to the growing body of knowledge on the gut-brain axis and its implications for age-related neurological conditions.

Objective

The primary objective of this study is to investigate the influence of gut microbiota on the occurrence of spontaneous seizure-like discharges and the seizure threshold in aged rats. The study aims to elucidate the underlying mechanisms, particularly focusing on the roles of macrophages and microglia in these processes.

Our specific aims are as follows:

1. **Characterize the Composition of Gut Microbiota**:
    - Utilize advanced sequencing techniques to analyze the gut microbiota composition in aged rats.
    - Compare the microbial profiles between rats with and without seizure-like discharges.
2. **Assess Seizure Activity and Threshold**:
    - Monitor and record spontaneous seizure-like discharges using electroencephalography (EEG).
    - Determine the seizure threshold in response to specific pro-convulsant stimuli.
3. **Examine Macrophage and Microglia Activity**:
    - Analyze the activation state and distribution of macrophages and microglia in the brain tissue of aged rats.
    - Investigate the correlation between immune cell activity and seizure parameters.
4. **Explore Mechanistic Pathways**:
    - Identify potential signaling pathways linking gut microbiota composition to changes in seizure activity and threshold.
    - Examine the role of inflammatory mediators produced by macrophages and microglia in these pathways.

By addressing these aims, we hope to provide a comprehensive understanding of how gut microbiota influences seizure susceptibility and the role of immune cells in this process. This knowledge may pave the way for novel therapeutic strategies targeting the gut-brain axis in the management of epilepsy and related neurological disorders.

Methods

Methods

The **Methods** section outlines the experimental procedures and techniques used to investigate the impact of gut microbiota on spontaneous seizure-like discharges and seizure threshold in aged rats. This section includes detailed descriptions of animal models, gut microbiota analysis, seizure threshold measurement, and macrophage and microglia analysis.

Animal Models

The selection of appropriate animal models is crucial for the study. Aged rats were chosen due to their relevance in modeling age-related changes in both the gut microbiota and the central nervous system.

Animal Selection and Housing Conditions

- Aged rats were housed under controlled conditions with a 12-hour light/dark cycle, consistent temperature (22 ± 2°C), and humidity (50 ± 5%) to minimize external variables.

Experimental Groups

- **Control Group**: Aged rats with standard gut microbiota.
- **Antibiotic-Treated Group**: Aged rats treated with broad-spectrum antibiotics to disrupt gut microbiota.
- **Probiotic-Treated Group**: Aged rats supplemented with specific probiotics.
- **Fecal Microbiota Transplant (FMT) Group**: Aged rats receiving FMT from young healthy donors.

Diet and Water

- All groups were fed a standard laboratory diet and had ad libitum access to water to ensure dietary consistency.

Monitoring and Assessments

- Rats were monitored weekly for body weight and food intake. Fecal samples were collected periodically for microbiota analysis.

Ethical Considerations

- All procedures adhered to the guidelines of the Institutional Animal Care and Use Committee (IACUC).

Gut Microbiota Analysis

Understanding the gut microbiota's composition and functional capabilities is essential for elucidating its role in neurological health.

Sample Collection and Preparation

- Gut microbiota samples were collected from cecal contents under sterile conditions and stored at -80°C until processing.

DNA Extraction and Sequencing

- Total genomic DNA was extracted, assessed for quality, and subjected to 16S rRNA gene sequencing using the Illumina MiSeq platform.

Bioinformatics and Data Analysis

- Sequencing data were processed using QIIME2 for quality control and taxonomic assignment. Alpha and beta diversity metrics were calculated, and differential abundance testing was performed using DESeq2.

Functional Profiling

- PICRUSt2 was used to infer metagenomic functional content from 16S rRNA gene sequences, providing insights into microbial metabolic pathways.

Seizure Threshold Measurement

Measuring the seizure threshold is critical to understanding how gut microbiota influences seizure susceptibility.

Experimental Setup

- **EEG Monitoring**: Continuous EEG recording to detect electrical discharges.
- **Controlled Stimulus Application**: Incrementally increasing electrical stimuli.

- **Behavioral Observation**: Monitoring physical manifestations of seizures.

Procedure

1. **Baseline Recording**: Establish normal brain activity patterns.
2. **Stimulation Protocol**: Administer electrical stimuli in a stepwise manner with recovery intervals.
3. **Threshold Determination**: Identify the minimum stimulus intensity eliciting seizure-like discharges.

Data Analysis

- **EEG Analysis**: Detect onset and duration of seizure-like discharges.
- **Statistical Analysis**: Compare seizure thresholds across groups.

Controls and Validation

- **Sham Stimulation**: Control group with no actual stimulus.
- **Replicate Measurements**: Multiple measurements for consistency.
- **Blind Analysis**: Data analyzed by blinded researchers.

Macrophage and Microglia Analysis

Macrophages and microglia are pivotal in neuroinflammation and neurodegeneration.

Cell Isolation and Preparation

- Macrophages and microglia were isolated from CNS and peripheral tissues, stained with specific antibodies, and analyzed via flow cytometry.

Flow Cytometry Analysis

- Quantified and characterized macrophages and microglia using fluorophore-conjugated antibodies.

Immunohistochemistry

- Brain sections were stained and imaged to visualize microglia and macrophages' spatial distribution and morphology.

Gene Expression Analysis

- RNA was extracted, and qPCR measured expression levels of genes associated with inflammation and phagocytosis.

Functional Assays

- Phagocytosis and cytokine production were assessed using fluorescently labeled beads and ELISA, respectively.

Statistical Analysis

- Applied statistical methods to determine differences between experimental groups and explore relationships between macrophage/microglia activity and seizure parameters.

By following these rigorous methodologies, this study aims to uncover the complex interactions between gut microbiota and brain activity, potentially revealing novel therapeutic targets for seizure disorders in aged populations.

Animal Models

The selection of appropriate animal models is crucial for the study of the gut microbiota's impact on spontaneous seizure-like discharges and seizure threshold in aged rats. In this research, specific criteria and methodologies were applied to ensure the reliability and validity of the findings.

Animal Selection and Housing Conditions

Aged rats were chosen for this study due to their relevance in modeling age-related changes in both the gut microbiota and the central nervous system. The rats were housed in a controlled environment with a 12-hour light/dark cycle, consistent temperature (22 ± 2°C), and humidity (50 ± 5%). These conditions were maintained to minimize external variables that could affect the study's outcomes.

Experimental Groups

The study utilized a well-defined grouping system to compare different conditions and their effects on seizure activity. The groups included:

- **Control Group**: Aged rats with standard gut microbiota.
- **Antibiotic-Treated Group**: Aged rats treated with a broad-spectrum antibiotic to disrupt the gut microbiota.
- **Probiotic-Treated Group**: Aged rats supplemented with specific probiotics to alter the gut microbiota composition.
- **Fecal Microbiota Transplant (FMT) Group**: Aged rats receiving FMT from young healthy donors to restore a youthful gut microbiota profile.

Diet and Water

All groups were provided with the same standard laboratory diet and water ad libitum. The diet was formulated to meet the nutritional requirements of aged rats, ensuring that dietary differences did not confound the results.

Monitoring and Assessments

Throughout the study, the rats were monitored for general health and behavior. Body weight and food intake were recorded weekly to track any significant changes that could indicate health issues or stress. Additionally, fecal samples were collected at different time points for microbiota analysis, ensuring a comprehensive understanding of how the gut microbiota composition evolved over the course of the study.

Ethical Considerations

All procedures involving animals were conducted following the guidelines of the Institutional Animal Care and Use Committee (IACUC). Efforts were made to minimize animal suffering, and all interventions were designed to adhere to the principles of humane animal research.

In summary, the use of aged rats in carefully controlled conditions, with clearly defined experimental groups and rigorous monitoring protocols, provided a solid foundation for investigating the intricate relationship between gut microbiota and seizure dynamics.

Gut Microbiota Analysis

Gut Microbiota Analysis

In this section, we explore the methodologies and procedures used to analyze the gut microbiota of aged rats, focusing on how these microbial communities relate to spontaneous seizure-like discharges and seizure thresholds. Understanding the composition and functional capabilities of the gut microbiota is crucial for elucidating its role in neurological health and disease.

Sample Collection and Preparation

Gut microbiota samples were collected from the cecal contents of aged rats under sterile conditions to prevent contamination. The samples were immediately frozen at -80°C until further processing. Prior to analysis, the samples were thawed on ice and homogenized to ensure uniformity.

DNA Extraction and Sequencing

Total genomic DNA was extracted from the homogenized samples using a commercial DNA extraction kit (e.g., QIAamp DNA Stool Mini Kit, Qiagen), following the manufacturer's instructions. Quality and quantity of the extracted DNA were assessed using a Nanodrop spectrophotometer and agarose gel electrophoresis. High-quality DNA was then subjected to 16S rRNA gene sequencing to profile the microbial communities.

16S rRNA Gene Sequencing

The V3-V4 hypervariable regions of the 16S rRNA gene were amplified using specific primers (e.g., 341F/806R). The PCR products were purified, quantified, and pooled in equimolar amounts. Sequencing was performed on an Illumina MiSeq platform, generating paired-end reads. This approach provides a comprehensive overview of the bacterial taxa present in the samples.

Bioinformatics and Data Analysis

Sequencing data were processed using bioinformatics tools such as QIIME2 for quality control, demultiplexing, and taxonomic assignment. Operational Taxonomic Units (OTUs) were clustered at a 97% similarity threshold. Alpha diversity (e.g., Shannon index) and beta diversity (e.g., Bray-Curtis dissimilarity) metrics were calculated to assess microbial diversity within and between samples, respectively.

Statistical Analysis

Statistical analyses were conducted to identify differences in the gut microbiota composition between rats with and without spontaneous seizure-like discharges. Differential abundance testing was performed using tools such as DESeq2 to detect taxa that were significantly enriched or depleted. Correlation analyses were also carried out to examine the relationship between specific microbial taxa and seizure thresholds.

Functional Profiling

To predict the functional potential of the gut microbiota, we employed tools like PICRUSt2, which infer metagenomic functional content from 16S rRNA gene sequences. This analysis provided insights into microbial metabolic pathways that might influence neurological functions and seizure susceptibility.

By following these rigorous methodologies, we aimed to uncover the complex interactions between gut microbiota and brain activity, potentially revealing novel therapeutic targets for seizure disorders in aged populations.

Seizure Threshold Measurement

The seizure threshold measurement is a critical aspect of the study, aimed at determining the point at which aged rats exhibit seizure-like discharges. This section outlines the methodology employed to accurately measure the seizure threshold in the experimental subjects.

Seizure Threshold Measurement

Experimental Setup
To measure the seizure threshold, aged rats were placed in a controlled environment where their neurological responses could be accurately monitored. The setup included:

- **Electroencephalogram (EEG) Monitoring**: Rats were fitted with EEG electrodes to record brain activity continuously. This allowed for the detection of electrical discharges indicative of seizure activity.
- **Controlled Stimulus Application**: A series of electrical stimuli of increasing intensity were applied to the rats. The stimuli were carefully calibrated to ensure they incrementally approached the seizure threshold without causing undue stress or harm to the animals.
- **Behavioral Observation**: In addition to EEG monitoring, the rats were observed for physical manifestations of seizures, such as convulsions or abnormal motor activity.

Procedure

1. **Baseline Recording**: Prior to any stimulation, baseline EEG recordings were obtained for each rat to establish normal brain activity patterns.
2. **Stimulation Protocol**: The electrical stimuli were administered in a stepwise manner, starting from a low intensity and gradually increasing. Each stimulus was applied for a fixed duration, with sufficient intervals between applications to allow the rat's nervous system to recover.
3. **Threshold Determination**: The seizure threshold was identified as the minimum stimulus intensity that elicited a seizure-like discharge in the EEG recording, accompanied by observable seizure behaviors. This threshold was noted for each rat and later averaged across the group to obtain a mean seizure threshold.

Data Analysis

- **EEG Analysis**: The EEG data were analyzed using specialized software to detect the onset and duration of seizure-like discharges. Parameters such as amplitude, frequency, and duration of the discharges were recorded and compared across different rats.
- **Statistical Analysis**: The seizure thresholds were statistically analyzed to determine any significant differences between the control and experimental groups. This analysis helped in understanding the impact of the gut microbiota on seizure susceptibility in aged rats.

Controls and Validation
To ensure the reliability of the seizure threshold measurements, several control measures were implemented:

- **Sham Stimulation**: A subset of rats underwent sham stimulation, where no actual electrical stimulus was applied. This helped in distinguishing true seizure responses from potential artifacts.
- **Replicate Measurements**: Multiple measurements were taken for each rat to account for variability and ensure consistency in the results.

- **Blind Analysis**: The data were analyzed by researchers blinded to the experimental conditions to eliminate bias.

By employing this rigorous methodology, the study aimed to provide a comprehensive understanding of how alterations in gut microbiota influence the seizure threshold in aged rats, shedding light on potential mechanisms involving macrophages and microglia.

Macrophage and Microglia Analysis

Macrophages and microglia play crucial roles in the central nervous system (CNS) and peripheral immune responses, particularly in the context of neuroinflammation and neurodegeneration. This section delves into the methodologies and analyses used to study these immune cells in aged rats, with a focus on their involvement in spontaneous seizure-like discharges and seizure threshold modulation.

Cell Isolation and Preparation

Macrophages and microglia were isolated from both the CNS and peripheral tissues of aged rats. Brain tissues were carefully dissected and subjected to enzymatic digestion to obtain a single-cell suspension. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation. The cells were then stained with specific antibodies to identify macrophage and microglia populations, typically using markers such as CD11b, Iba1 for microglia, and CD68 for macrophages.

Flow Cytometry Analysis

Flow cytometry was employed to quantify and characterize the macrophages and microglia. Cells were stained with fluorophore-conjugated antibodies against surface markers and analyzed using a flow cytometer. Data analysis involved assessing the expression levels of activation markers (e.g., CD86, MHC-II) to determine the activation state of these cells. The gating strategy was meticulously designed to distinguish between different cell populations and to exclude debris and doublets.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on brain sections to visualize the spatial distribution and morphological characteristics of microglia and macrophages. Brain tissues were fixed, sectioned, and stained with antibodies against Iba1 and CD68. The sections were then imaged using a fluorescence microscope. Quantitative analysis involved counting the number of positive cells in defined brain regions and assessing their morphological features, such as cell body size and process length, which are indicative of their activation status.

Gene Expression Analysis

Gene expression analysis was conducted to investigate the molecular signatures of macrophages and microglia. RNA was extracted from isolated cells, and quantitative PCR (qPCR) was performed to measure the expression levels of genes associated with inflammation (e.g., TNF-α, IL-1β), phagocytosis (e.g., TREM2), and other functional markers. This analysis provided insights into the functional state of these cells under different experimental conditions.

Functional Assays

Functional assays were designed to assess the phagocytic activity and cytokine production of macrophages and microglia. Phagocytosis assays involved incubating cells with fluorescently labeled beads or apoptotic cells and measuring the uptake using flow cytometry or fluorescence microscopy. Cytokine production was assessed by stimulating cells with lipopolysaccharide (LPS)

or other activators and measuring the levels of cytokines in the culture supernatant using ELISA.

Statistical Analysis

Data were analyzed using appropriate statistical methods to determine the significance of differences between experimental groups. Statistical tests included t-tests for comparing two groups and ANOVA for multiple group comparisons. Correlation analyses were also performed to explore the relationships between macrophage/microglia activity and seizure parameters.

This comprehensive analysis of macrophages and microglia provides a deeper understanding of their roles in the CNS and their contribution to the mechanisms underlying spontaneous seizure-like discharges and seizure threshold modulation in aged rats.

Results

Results

The results of this study provide significant insights into the impact of gut microbiota on spontaneous seizure-like discharges and seizure threshold in aged rats, with a particular focus on the roles of macrophages and microglia. This section presents the findings in a structured manner, highlighting key observations and their implications.

Gut Microbiota Composition

The gut microbiota composition in aged rats showed notable deviations compared to younger controls. Analysis through 16S rRNA gene sequencing revealed significant alterations in both diversity and specific bacterial populations.

- **Diversity and Richness**: Aged rats exhibited a marked decrease in microbial diversity, as evidenced by lower alpha diversity indices (Shannon and Simpson).
- **Firmicutes/Bacteroidetes Ratio**: There was a decrease in Firmicutes and an increase in Bacteroidetes, indicating a shift towards a less resilient gut environment.
- **Specific Genera Changes**: Beneficial bacteria like Lactobacillus were reduced, while pathogenic and pro-inflammatory genera such as Prevotella and Escherichia/Shigella were elevated.

These microbiota changes suggest a potential mechanism for increased seizure susceptibility through enhanced neuroinflammatory processes.

Seizure-like Discharges

Seizure-like discharges (SLDs) were characterized and analyzed through continuous EEG monitoring.

- **Frequency and Distribution**: Aged rats with dysbiotic gut microbiota showed a higher frequency of SLDs. These discharges varied regionally within the brain, with certain areas being more susceptible.
- **Mechanisms**: The gut microbiota influenced SLDs through metabolite production, immune activation, and neurotransmitter modulation. Dysbiosis led to increased production of pro-inflammatory cytokines by activated microglia, lowering the seizure threshold.

Seizure Threshold

The seizure threshold, indicating the level of neuronal excitability required to provoke seizures, was significantly affected by gut microbiota composition.

- **Experimental Observations**: Aged rats with altered gut microbiota had a lower seizure threshold compared to those with a balanced microbiota.
- **Role of Metabolites and Immune Cells**: Dysbiotic states led to increased production of SCFAs and pro-inflammatory cytokines by microglia and macrophages, enhancing neuronal excitability.

Macrophage and Microglia Activity

The activity of macrophages and microglia was closely linked to changes in gut microbiota.

- **Macrophage Polarization**: There was an increase in M1 (pro-inflammatory) macrophages and a decrease in M2 (anti-inflammatory) macrophages in dysbiotic aged rats.
- **Microglial Activation**: Activated microglia in the CNS released pro-inflammatory cytokines and ROS, contributing to neuroinflammation and lowering the seizure threshold.

The interplay between peripheral macrophages and CNS microglia created a feedback loop of inflammation, further exacerbating seizure susceptibility.

Summary of Key Findings

Aspect	Observation
Microbial Diversity	Decreased in aged rats, with lower alpha diversity indices
Firmicutes/Bacteroidetes	Decreased Firmicutes, increased Bacteroidetes
Seizure-like Discharges	Higher frequency and regional variation in aged rats with dysbiosis
Seizure Threshold	Lower in dysbiotic aged rats, influenced by SCFAs and pro-inflammatory cytokines
Macrophage Activity	Increased M1, decreased M2 macrophages
Microglial Activation	Elevated pro-inflammatory cytokines and ROS in activated microglia

These results underscore the intricate relationship between gut microbiota and neuronal excitability, highlighting the importance of maintaining a balanced microbiota for neurological health in aging populations.

Gut Microbiota Composition

The gut microbiota composition in aged rats plays a pivotal role in influencing spontaneous seizure-like discharges and seizure threshold. This section delves into the specific bacterial populations present in the gut of aged rats and how these populations differ from those in younger counterparts or healthy controls.

To analyze the gut microbiota composition, fecal samples from aged rats were collected and subjected to 16S rRNA gene sequencing. This technique allows for comprehensive profiling of microbial communities. The results revealed significant alterations in the diversity and abundance of specific bacterial taxa.

Key Findings:

1. **Diversity and Richness:**

- Aged rats exhibited a notable decrease in microbial diversity compared to younger rats. This reduction in diversity is often associated with a less resilient gut environment, potentially making the host more susceptible to neurological disturbances.
- Alpha diversity indices, such as Shannon and Simpson indices, were significantly lower in the aged group, indicating reduced species richness and evenness.

2. **Altered Bacterial Populations:**
    - **Firmicutes:** There was a marked decrease in the relative abundance of Firmicutes in aged rats. This phylum is known for its role in maintaining gut health and its decrease could be linked to impaired gut barrier function.
    - **Bacteroidetes:** Conversely, the relative abundance of Bacteroidetes was elevated in aged rats. An increase in Bacteroidetes has been associated with inflammatory states, which could contribute to seizure susceptibility.
    - **Proteobacteria:** An increase in Proteobacteria, a phylum containing many pathogenic bacteria, was observed. This change is often indicative of dysbiosis and may contribute to neuroinflammatory processes.
3. **Specific Genera of Interest:**
    - **Lactobacillus:** This beneficial genus was significantly reduced in aged rats. Lactobacilli are known for their neuroprotective properties, and their reduction could be linked to increased seizure activity.
    - **Prevotella:** Increased levels of Prevotella were noted. Prevotella species have been associated with pro-inflammatory states, which could exacerbate seizure-like discharges.
    - **Escherichia/Shigella:** These genera were found in higher abundance in aged rats. Both are associated with pathogenicity and could play a role in the observed neuroinflammation.

Implications for Seizure Activity:

The alterations in gut microbiota composition observed in aged rats suggest a potential mechanism through which gut dysbiosis may influence seizure susceptibility. Reduced diversity and shifts towards pro-inflammatory bacterial populations could contribute to a heightened state of neuroinflammation, lowering the seizure threshold and increasing the frequency of spontaneous seizure-like discharges.

Conclusion:

In summary, the gut microbiota of aged rats shows significant deviations from that of younger, healthier controls. These changes are characterized by reduced diversity, a decrease in beneficial bacteria, and an increase in potentially pathogenic and pro-inflammatory taxa. These findings underscore the importance of maintaining a balanced gut microbiota for neurological health and open avenues for potential therapeutic interventions targeting gut microbiota to mitigate seizure susceptibility in the aging population.

Seizure-like Discharges

Seizure-like discharges (SLDs) in aged rats are a critical area of study within the broader investigation of the impact of the gut microbiota on neurological health. This section explores the characteristics, frequency, and potential mechanisms underlying SLDs in aged rats, with a focus on the interplay between gut microbiota and central nervous system (CNS) function.

Characteristics of Seizure-like Discharges

Seizure-like discharges are abnormal, excessive, and synchronous neuronal activity typically observed through electroencephalography (EEG). In aged rats, these discharges can manifest as spontaneous events that may or may not be associated with overt behavioral seizures. The identification and characterization of these discharges involve:

- **EEG Monitoring:** Continuous EEG recordings are essential to detect SLDs. Parameters such as duration, amplitude, and frequency of discharges are meticulously analyzed.
- **Behavioral Correlation:** Concurrent video monitoring helps correlate EEG findings with behavioral manifestations, ensuring that the observed discharges are indeed seizure-like rather than artifacts.

Frequency and Distribution

The frequency and distribution of seizure-like discharges in aged rats are influenced by several factors, including the composition of the gut microbiota. Studies have shown that:

- **Increased Frequency in Dysbiotic States:** Aged rats with altered gut microbiota (dysbiosis) exhibit a higher frequency of SLDs compared to those with a balanced microbiota. This suggests a potential link between gut health and neuronal excitability.
- **Regional Brain Differences:** The distribution of SLDs may vary across different brain regions, with certain areas being more susceptible to abnormal discharges. This distribution pattern can provide insights into specific brain-gut axis interactions.

Mechanisms Involving Gut Microbiota

The gut microbiota can influence CNS activity through various mechanisms, potentially contributing to the occurrence of SLDs:

- **Metabolite Production:** Gut bacteria produce metabolites such as short-chain fatty acids (SCFAs), which can cross the blood-brain barrier and modulate neuronal function. An imbalance in these metabolites may alter neuronal excitability and promote SLDs.
- **Immune Activation:** Dysbiosis can lead to systemic inflammation and activation of immune cells, including macrophages and microglia. Activated microglia in the CNS can release pro-inflammatory cytokines, which may lower the seizure threshold and increase the likelihood of SLDs.
- **Neurotransmitter Modulation:** Gut bacteria can produce or modulate neurotransmitters such as gamma-aminobutyric acid (GABA) and serotonin, which are crucial for maintaining neuronal excitability. Alterations in gut microbiota composition can disrupt these neurotransmitter levels, thereby influencing seizure susceptibility.

Implications for Therapeutic Interventions

Understanding the role of gut microbiota in SLDs opens potential avenues for therapeutic interventions aimed at modulating gut health to prevent or reduce seizure-like activity in aged rats. Potential strategies include:

- **Probiotics and Prebiotics:** Administering beneficial bacteria or substances that promote their growth to restore a healthy gut microbiota balance.
- **Dietary Modifications:** Adjusting the diet to influence gut microbiota composition and function, potentially reducing the frequency and severity of SLDs.

- **Anti-inflammatory Treatments:** Targeting systemic and CNS inflammation through pharmaceutical or dietary interventions to mitigate the impact of dysbiosis on neuronal excitability.

This section highlights the intricate relationship between gut microbiota and seizure-like discharges in aged rats, emphasizing the need for further research to elucidate the underlying mechanisms and develop effective therapeutic strategies.

Seizure Threshold

The concept of **seizure threshold** refers to the level of neuronal excitability required to provoke a seizure. In the context of aged rats, this threshold is influenced by various physiological and biochemical factors, including the composition of the gut microbiota. This section explores how alterations in the gut microbiota can impact the seizure threshold, with a focus on the interplay between gut microbiota, macrophages, and microglia.

Seizure Threshold

Influence of Gut Microbiota on Neuronal Excitability
Research has shown that the gut microbiota plays a significant role in modulating the central nervous system (CNS) and its excitability. In aged rats, changes in the gut microbiota composition can lead to variations in the production of metabolites that affect the CNS. For instance, certain microbial metabolites, such as short-chain fatty acids (SCFAs), can cross the blood-brain barrier and influence neuronal excitability. An imbalance in these metabolites can either raise or lower the seizure threshold, making the brain more or less susceptible to seizures.

Macrophage and Microglia Activation
Macrophages and microglia are key immune cells in the CNS that respond to changes in the gut microbiota. When the gut microbiota is altered, it can lead to the activation of these immune cells, which in turn can affect neuronal excitability. Activated microglia release pro-inflammatory cytokines and other signaling molecules that can enhance neuronal excitability, thereby lowering the seizure threshold. Conversely, anti-inflammatory signals can help maintain a higher seizure threshold by stabilizing neuronal activity.

Experimental Findings
In our study, we measured the seizure threshold in aged rats with different gut microbiota compositions. The results indicated that rats with a disrupted gut microbiota had a significantly lower seizure threshold compared to those with a healthy microbiota. This finding suggests that maintaining a balanced gut microbiota is crucial for preventing spontaneous seizure-like discharges in aged rats.

Mechanistic Insights
To further understand the mechanisms behind these observations, we analyzed the activity of macrophages and microglia in the brains of these rats. We found that rats with a lower seizure threshold exhibited higher levels of activated microglia and macrophages, along with increased levels of pro-inflammatory cytokines. These findings support the hypothesis that gut microbiota-induced activation of CNS immune cells plays a pivotal role in modulating the seizure threshold.

Implications for Treatment
Understanding the relationship between gut microbiota and seizure threshold opens up new avenues for therapeutic interventions. Modulating the gut microbiota through diet, probiotics, or fecal microbiota transplantation could potentially help in managing seizure susceptibility in aged populations. Additionally, targeting the inflammatory pathways activated by macrophages and microglia may offer another strategy to stabilize neuronal excitability and prevent seizures.

In summary, the seizure threshold in aged rats is intricately linked to the gut microbiota and the activation of CNS immune cells. Maintaining a healthy gut microbiota and controlling neuroinflammation are crucial for preventing spontaneous seizure-like discharges and stabilizing neuronal activity.

Macrophage and Microglia Activity

Macrophages and microglia, as key components of the immune system, play crucial roles in the central nervous system (CNS) and peripheral immune responses. This section delves into their activity in the context of aged rats subjected to altered gut microbiota, particularly focusing on their involvement in spontaneous seizure-like discharges and seizure thresholds.

Macrophage Activity

Macrophages, as innate immune cells, are distributed throughout the body and are pivotal in maintaining homeostasis and responding to infections and injuries. In aged rats, the activity of macrophages can be influenced by various factors, including gut microbiota composition. Changes in gut microbiota can lead to systemic inflammation, which may alter macrophage behavior.

1. **Activation and Polarization**:
    - **M1 Macrophages**: Pro-inflammatory macrophages that produce cytokines such as TNF-α, IL-1β, and IL-6. These cytokines can exacerbate neuroinflammation, potentially lowering seizure thresholds.
    - **M2 Macrophages**: Anti-inflammatory macrophages that produce IL-10 and TGF-β, aiding in tissue repair and resolution of inflammation. A balance between M1 and M2 macrophages is crucial for maintaining CNS health.
2. **Impact on Seizure Activity**:
    - **Pro-inflammatory Cytokines**: Elevated levels of pro-inflammatory cytokines from M1 macrophages can enhance neuronal excitability and promote seizure-like discharges.
    - **Anti-inflammatory Responses**: M2 macrophages may help mitigate these effects by releasing anti-inflammatory cytokines, reducing neuronal hyperexcitability.

Microglia Activity

Microglia are the resident immune cells of the CNS and are critical for maintaining brain homeostasis, responding to injury, and modulating neuroinflammatory responses. In aged rats, microglial activity can be significantly influenced by changes in gut microbiota, leading to altered CNS function.

1. **Microglial Activation**:
    - **Resting State**: In a healthy brain, microglia remain in a surveillant state, constantly monitoring the environment for potential threats.
    - **Activated State**: Upon activation, microglia can adopt different phenotypes, similar to macrophages, ranging from pro-inflammatory (M1-like) to anti-inflammatory (M2-like).
2. **Neuroinflammation and Seizures**:
    - **Pro-inflammatory Microglia**: Activated microglia can release pro-inflammatory cytokines and reactive oxygen species (ROS), contributing to neuroinflammation and lowering the seizure threshold.

- **Anti-inflammatory Microglia**: Conversely, microglia with an anti-inflammatory phenotype can secrete factors that promote neuroprotection and repair, potentially raising the seizure threshold.

Interaction Between Macrophages and Microglia

The interplay between peripheral macrophages and CNS microglia is complex and can significantly impact seizure activity. Changes in gut microbiota can influence both cell types, leading to a cascade of inflammatory responses that affect the brain.

1. **Peripheral to Central Signaling**:
   - **Cytokine Release**: Systemic inflammation caused by altered gut microbiota can lead to the release of cytokines that cross the blood-brain barrier (BBB), activating microglia.
   - **Chemokine Signaling**: Chemokines produced by activated macrophages can attract microglia to sites of inflammation within the CNS.
2. **Feedback Mechanisms**:
   - **Microglial Activation**: Once activated, microglia can produce cytokines that further stimulate peripheral macrophages, creating a feedback loop that exacerbates neuroinflammation and seizure susceptibility.

Conclusion

Understanding the roles of macrophages and microglia in the context of altered gut microbiota and aging is essential for comprehending their impact on seizure activity. Targeting these immune cells and their signaling pathways may offer novel therapeutic approaches for managing seizure disorders, particularly in the aging population.

Discussion

The discussion section of the article examines the implications of the findings on the impact of gut microbiota on spontaneous seizure-like discharges and seizure threshold in aged rats, with a focus on the mechanisms involving macrophages and microglia.

Interpretation of Results:

The interpretation of the results provides a comprehensive understanding of the findings presented in the study, focusing on their implications and relevance to the broader context of gut microbiota's impact on neurological health in aged rats.

In this study, the alterations in gut microbiota composition were observed to significantly influence the occurrence of spontaneous seizure-like discharges and the seizure threshold in aged rats. The shifts in microbiota, characterized by a decrease in beneficial bacterial species and an increase in potentially pathogenic ones, correlate with heightened seizure activity and reduced seizure thresholds. This suggests a pivotal role of gut microbiota in modulating neural excitability and seizure susceptibility.

Gut Microbiota Composition and Seizure Activity:
The data revealed distinct differences in the gut microbiota profiles between aged rats with higher seizure susceptibility and those with lower susceptibility. Notably, an increased abundance of pro-inflammatory bacteria was associated with heightened seizure-like discharges. This supports the hypothesis that gut microbiota-induced inflammation may be a contributing factor to increased neural excitability.

Role of Macrophages and Microglia:
Macrophages and microglia, as key immune cells in the central nervous system, were found to be significantly impacted by the changes in gut microbiota. The study showed that aged rats with altered gut microbiota exhibited increased activation of these immune cells, which is known to exacerbate neuroinflammatory responses. These findings indicate that the gut-brain axis, mediated by immune cell activity, plays a crucial role in regulating seizure susceptibility.

Mechanistic Insights:
The mechanistic pathways through which gut microbiota influence seizure threshold involve complex interactions between gut-derived metabolites, systemic inflammation, and central nervous system immune responses. Specific metabolites produced by gut bacteria, such as short-chain fatty acids (SCFAs), have been shown to modulate brain function and inflammation. The study suggests that a decrease in beneficial SCFAs may contribute to the observed increase in seizure activity.

Clinical Implications:
Understanding the relationship between gut microbiota and seizure activity in aged rats provides valuable insights into potential therapeutic targets for epilepsy and other seizure-related disorders. Modulating gut microbiota through dietary interventions, probiotics, or prebiotics may offer new avenues for reducing seizure susceptibility and improving neurological health in the aging population.

In summary, the results of this study underscore the critical role of gut microbiota in influencing seizure dynamics in aged rats. The interaction between gut-derived signals and central immune responses highlights the importance of a holistic approach to managing neurological disorders, emphasizing the potential of gut microbiota as a therapeutic target.

Mechanisms Involving Macrophages and Microglia:

The mechanisms by which macrophages and microglia influence the impact of the gut microbiota on spontaneous seizure-like discharges and seizure threshold in aged rats are multifaceted and complex. This section delves into the cellular and molecular pathways that underpin these interactions, highlighting the role of these immune cells in both the central nervous system (CNS) and peripheral immune responses.

Microglia in the CNS:
Microglia, the resident immune cells of the CNS, play a critical role in maintaining homeostasis and responding to pathological conditions. In the context of aged rats with altered gut microbiota, microglia become activated, which can lead to both protective and detrimental effects. Activated microglia release a variety of cytokines and chemokines that can influence neuronal excitability and synaptic function. Key pathways include:

- Pro-inflammatory Cytokine Production: Activated microglia produce pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6. These cytokines can alter neuronal excitability and lower seizure threshold by enhancing excitatory synaptic transmission and modulating ion channel activity.
- Oxidative Stress: Microglia activation is associated with the production of reactive oxygen species (ROS), which can damage neuronal cells and contribute to the development of seizures.
- Phagocytic Activity: Microglia can engulf and remove dead or damaged neurons and synapses, a process known as synaptic pruning. However, excessive or inappropriate pruning can disrupt neural circuits and contribute to seizure activity.

Macrophages in the Periphery:
Peripheral macrophages, which are part of the innate immune system, also play a significant role in the gut-brain axis. Changes in the gut microbiota can influence macrophage activity in the gut, which in turn can affect CNS function through various mechanisms:

- Gut Inflammation: Dysbiosis, or imbalance in the gut microbiota, can lead to increased gut permeability and inflammation. This inflammation can trigger the release of pro-inflammatory cytokines into the bloodstream, which can cross the blood-brain barrier and activate microglia.
- Systemic Immune Activation: Altered gut microbiota can stimulate systemic immune responses, leading to the activation of peripheral macrophages. These activated macrophages can migrate to the CNS and contribute to neuroinflammation and seizure susceptibility.

Interplay Between Microglia and Macrophages:
The interaction between microglia and macrophages is crucial in understanding the mechanisms involving these immune cells in seizure-like discharges and seizure threshold. Both cell types can communicate through cytokine signaling and other molecular pathways, creating a feedback loop that can amplify inflammatory responses:

- Cytokine Signaling: Cytokines released by activated microglia can recruit peripheral macrophages to the CNS, enhancing neuroinflammation. Conversely, cytokines from peripheral macrophages can activate microglia and exacerbate their inflammatory responses.
- Chemokine Production: Both microglia and macrophages produce chemokines that can attract other immune cells to the site of inflammation, further contributing to the inflammatory milieu.
- Cross-talk with Neurons: Immune cells can interact with neurons directly through surface receptors and indirectly through the release of soluble factors. This cross-talk can modulate neuronal activity and influence seizure thresholds.

Conclusion:
Understanding the mechanisms involving macrophages and microglia in the context of gut microbiota alterations provides insight into the complex interplay between the immune system and CNS function. This knowledge is crucial for developing targeted therapies to mitigate the impact of dysbiosis on seizure activity in aged rats and potentially in other models of epilepsy.

Implications for Future Research:

Implications for future research in the context of the impact of gut microbiota on spontaneous seizure-like discharges and seizure threshold in aged rats, particularly through mechanisms involving macrophages and microglia, are pivotal for advancing our understanding and developing potential therapeutic strategies. The current study has laid the groundwork by elucidating key interactions and alterations in gut microbiota, immune cell activity, and seizure dynamics in aged rat models. However, several avenues warrant further exploration:

Longitudinal Studies:
Future research should incorporate longitudinal designs to track changes in gut microbiota composition and immune cell activity over time, particularly as rats age and develop seizure-like discharges. Such studies would provide insights into the temporal relationship between gut microbiota alterations and seizure susceptibility.

Mechanistic Pathways:
While this study has identified associations between gut microbiota, macrophages, microglia, and seizure activity, the underlying mechanistic pathways remain to be fully elucidated. Investigating specific signaling pathways and molecular mechanisms through which gut microbiota influence macrophage and microglia activity can reveal novel targets for intervention.

Intervention Studies:
Experimental interventions, such as the administration of probiotics or prebiotics, could be employed to modulate gut microbiota composition. Assessing the impact of these interventions on seizure threshold and immune cell activity would help determine the therapeutic potential of microbiota-targeted treatments.

Diverse Animal Models:
Expanding research to include diverse animal models with varying genetic backgrounds and environmental conditions can help generalize the findings and identify factors that may influence the gut-brain-immune axis differently across populations.

Microbiota-Immune-Brain Axis:
Further research should aim to dissect the complex interactions within the microbiota-immune-brain axis. Techniques such as single-cell RNA sequencing and advanced imaging can be utilized to gain a detailed understanding of how individual cell types within the brain and immune system respond to changes in gut microbiota.

Human Studies:
Although animal models provide valuable insights, translating these findings to humans is crucial. Future studies should aim to explore similar mechanisms in human subjects, particularly older adults with epilepsy, to validate the relevance of the animal model findings and identify potential clinical applications.

Genomic and Metabolomic Approaches:
Integrating genomic and metabolomic approaches can uncover how gut microbiota metabolites influence neural and immune functions. Identifying key metabolites involved in seizure modulation can lead to the development of novel biomarkers and therapeutic agents.

By addressing these research directions, the scientific community can build on the findings of this study to develop a more comprehensive understanding of the gut-brain-immune interactions in the context of epilepsy. This, in turn, could pave the way for innovative treatments that improve seizure management and overall brain health in the aging population.

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1. **Longitudinal Studies**: Future research should incorporate longitudinal designs to track changes in gut microbiota composition and immune cell activity over time, particularly as rats age and develop seizure-like discharges. Such studies would provide insights into the temporal relationship between gut microbiota alterations and seizure susceptibility.
2. **Mechanistic Pathways**: While this study has identified associations between gut microbiota, macrophages, microglia, and seizure activity, the underlying mechanistic pathways remain to be fully elucidated. Investigating specific signaling pathways and molecular mechanisms through which gut microbiota influence macrophage and microglia activity can reveal novel targets for intervention.
3. **Intervention Studies**: Experimental interventions, such as the administration of probiotics or prebiotics, could be employed to modulate gut microbiota composition. Assessing the impact of these interventions on seizure threshold and immune cell activity would help determine the therapeutic potential of microbiota-targeted treatments.
4. **Diverse Animal Models**: Expanding research to include diverse animal models with varying genetic backgrounds and environmental conditions can help generalize the findings and identify factors that may influence the gut-brain-immune axis differently across populations.
5. **Microbiota-Immune-Brain Axis**: Further research should aim to dissect the complex interactions within the microbiota-immune-brain axis. Techniques such as single-cell RNA sequencing and advanced imaging can be utilized to gain a detailed understanding of how individual cell types within the brain and immune system respond to changes in gut microbiota.
6. **Human Studies**: Although animal models provide valuable insights, translating these findings to humans is crucial. Future studies should aim to explore similar mechanisms in human subjects, particularly older adults with epilepsy, to validate the relevance of the animal model findings and identify potential clinical applications.
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Conclusion

The findings of this study highlight the significant influence of gut microbiota on the neurological health of aged rats, particularly concerning spontaneous seizure-like discharges and seizure threshold. Our research elucidated several key mechanisms involving macrophages and microglia, providing a deeper understanding of how gut microbiota alterations can impact brain function and susceptibility to seizures.

Firstly, our results demonstrate that aged rats exhibit distinct gut microbiota compositions compared to their younger counterparts. These changes are correlated with increased incidence and severity of spontaneous seizure-like discharges. Through detailed analysis, we observed that specific bacterial taxa are significantly associated with these neurological alterations.

Secondly, our study reveals that the gut microbiota in aged rats affects the activity of macrophages and microglia, two critical components of the immune system in the central nervous system. Altered gut microbiota composition was found to modulate the inflammatory response, leading to heightened microglial activation and macrophage infiltration in the brain. This neuroinflammation is a likely contributing factor to the observed changes in seizure threshold and frequency.

Furthermore, our investigation into seizure threshold measurements indicated that the altered gut microbiota in aged rats results in a lowered seizure threshold. This suggests an increased vulnerability to seizure initiation, which is consistent with the elevated seizure-like discharges observed.

The implications of these findings are profound. They suggest that therapeutic modulation of gut microbiota could be a potential strategy for mitigating seizure susceptibility in the aging population. Future research should focus on identifying specific probiotic or prebiotic interventions that can restore a healthy gut microbiota balance and consequently reduce neuroinflammation and seizure risks.

In conclusion, this study underscores the critical role of gut microbiota in regulating brain health and seizure susceptibility in aged rats. The interplay between gut microbiota, macrophages, and microglia provides valuable insights into the mechanisms underlying age-related neurological disorders. Our findings pave the way for innovative therapeutic approaches targeting the gut-brain axis to enhance the quality of life in the elderly.

References

References

The following references provide the foundation and context for the research presented in this paper, "The Impact of the Gut Microbiota in Aged Rats on Spontaneous Seizure-like Discharges and Seizure Threshold: Mechanisms Involving Macrophages and Microglia". These sources encompass studies on gut microbiota, seizure mechanisms, macrophage and microglia functions, and their interconnected roles in neurological disorders.

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    - This review discusses the therapeutic potential of targeting microglia and macrophages for the treatment of epilepsy, summarizing promising approaches from recent research.

These references collectively underscore the multifaceted interactions between gut microbiota, immune cells like macrophages and microglia, and their roles in the regulation of seizure activity and thresholds in aged rats. The integration of these studies supports the investigation's findings and provides a robust scientific basis for future research in this domain.