Spinal cord injury and gut microbiota: A review

Yingli Jing, Fan Bai, Yan Yu

PII: S0024-3205(20)31618-0

DOI: https://doi.org/10.1016/j.lfs.2020.118865

Reference: LFS 118865

To appear in: *Life Sciences*

Received date: 3 July 2020

Revised date: 26 November 2020

Accepted date: 2 December 2020

Please cite this article as: Y. Jing, F. Bai and Y. Yu, Spinal cord injury and gut microbiota: A review, *Life Sciences* (2020), https://doi.org/10.1016/j.lfs.2020.118865

Spinal cord injury and gut microbiota: A review

Yingli Jing[a,b,c,d], Fan Bai[a,b,c,d], Yan Yu[a,b,c,d*]

[a]China Rehabilitation Science Institute, Beijing 100068, China

[b]China Rehabilitation Research Center, Beijing 100068, China

[c]Beijing Key Laboratory of Neural Injury and Rehabilitation, Beijing 100068, China

[d]Center of Neural Injury and Repair, Beijing Institute for Brain Disorders, Beijing 100068, China

* Corresponding author at: Number 10, Jiao men North Road, Feng tai District, Beijing 100068, China. E-mail address: yuyancrrc@163.com.

Key words: spinal cord injury, gut microbiota, "microbiota-gut-brain" axis

Word count: 5728

Figure count: 2

Abstract

After spinal cord injury (SCI), intestinal dysfunction has a serious impact on physical and mental health, quality of life, and social participation. Recent data from rodent and human studies indicated that SCI causes gut dysbiosis. Remolding gut microbiota could be beneficial for the recovery of intestinal function and motor function after SCI. However, few studies have explored SCI with focus on the gut microbiota. This review will demonstrate the relationship of SCI and its complications with gut microbiota with focus on "microbiota-gut-brain" axis. In this review, the complications following SCI, including intestinal dysfunction, anxiety and depression, metabolic disorders, and neuropathic pain, are directly or indirectly related to gut dysbiosis, which may be mediated by "gut-brain" interactions. Furthermore, we discuss the research strategies that can be beneficial in this regard, including germ-free animals, fecal microbiota transplantation, probiotics, phages, and brain imaging techniques. The current microbial research has shifted from descriptive to mechanismal perspective, and future research using new technologies may further demonstrate the pathophysiological mechanism of association of SCI with gut microbiota, elucidate the mode of interaction of gut microbiota and hosts, and help develop personalized targeted microbiota therapies and drugs based on microbiota or corresponding metabolites.

Key words: spinal cord injury; complications; gut microbiota; microbiota-gut-brain axis

Background

Spinal cord injury (SCI) results in serious disabling conditions; about 90% of SCIs are caused by trauma [1,2]. Enhancing tissue repair and neural plasticity after SCI has always been difficult in the field of neuroscience, and there is no effective treatment for neural regeneration after SCI

[3,4]. Clinical interventions and treatments for SCI largely depend on the treatment of the symptoms and achievement of maximum functional recovery of the spared neurological function. Because of the loss of the regulatory and control function of the brain and/or upper spinal cord on the limbs and visceral organs below the injury level, sensory, motor, and multiple organ dysfunctions occur [5-7]. In 27–41% of adult patients with SCI, symptoms of gastrointestinal dysfunction that are particularly prominent and need treatment occur [8]. Surveys among the SCI population prioritize recovery of intestinal function above the ability to walk [9, 10]. With advancements in the field of medicine, the 40-year survival rate of patients with paraplegia and quadriplegia has improved significantly [11]. Intestinal dysfunction is considered to be an important factor that affects the physical and mental health of patients with SCI [12, 13], and its management has become a problem that directly affects the quality of life of patients. Recently, gut microbiota has become a research hotspot, and therapeutic strategies targeting the gut microbiome are highly anticipated. However, there are few studies on SCI that focus on gut microbiota; therefore, substantial efforts should be devoted to carry out extensive research on this topic. This review revolves about the relationship of SCI and associated complications with gut microbiota, focusing on "microbiota-gut-brain axis" and associated research strategies such as germ-free animals, fecal microbiota transplantation, probiotics, bacteriophage and brain imaging .

SCI and gut dysbiosis

SCI destroys the autonomic nervous system; consequently, injured descending axonal projections can no longer innervate motor neuron pools below the injury level, and injured ascending axonal projections can no longer transmit appropriate sensory information to the brain [14]. After SCI, the sympathetic control of the stomach, small intestine, and colon is lost, which

leads to the imbalance of autonomic tone and causes acute and persistent gastrointestinal dysfunction followed by deficits in colonic motility, mucosal secretion, vascular tone, and immune function [15, 16]. Gut microbiota may be an important factor associated with many pathological manifestations of imbalanced intestinal and immune functions.

At present, there are few studies on gut microbiota after SCI. In 2016, Bilgi Gungor et al. [17] designed a clinical study including 15 patients with upper motor neuron syndrome SCI, 15 patients with lower motor neuron syndrome SCI, and 10 healthy controls; they applied 16S rDNA technique to sequence and analyze the gut microbiota in fecal samples and found that the number of butyrate producing colonies was specifically reduced in SCI patients when compared to healthy subjects. In our previous clinical study, a total of 43 male patients with chronic traumatic complete SCI and 23 healthy male adults were enrolled. We collected the clinical dates and fresh stool speciments of the subjects for 16S rDNA gene sequencing. The results showed that the composition of microbiota was altered after SCI, which was related to blood biochemical indices and neurogenic intestinal dysfunction. Our data also show that there is a significant difference between the gut microbial composition of patients with cervical SCI and that of patients with thoracolumbar SCI [18]. In addition, age and sex may affect the identification of disease-related bacterial characteristics [19, 20]. In subsequent clinical and preclinical SCI studies, age and sex should be considered as variables. Clinical data showed that the abundance and composition of gut microbiota were altered after SCI, but the causal relationship or correlation between the alteration of gut microbiota and the recovery of locomotory function was not clear.

In 2016, researchers from Ohio State University found that SCI caused gut dysbiosis, which would aggravate neurological damage and reduce the repair of locomotory function. While,

probiotic-based (VSL#3) interventions could partially reverse the damage and contribute to the repair of motor function [21], recent data from our study based on mouse models of SCI indicated that melatonin could remodel gut microbiota and directly or indirectly promote the repair of motor function [22]. Additionally, these two articles presented a view that the use of broad-spectrum antibiotics is not conducive to the recovery of motor function. However, early surgical treatment or anti-infection therapy of SCI would need the antibiotic administration. Studies have shown that lung and wound infections in patients with SCI are associated with poor neurological recovery [23]. Therefore, it is worth evaluating further whether early use of antibiotics will affect the prognosis of SCI. Kigel et al. found that the gut microbial imbalance after SCI was related to a significant change in the relative proportion of immune cells in gut associated lymphoid tissue (GALT) [21]. O'Connor et al. found that the high expression levels of inflammatory cytokines (IL-1β, IL-12, MIP-2) were related to the relative abundance of specific types of gut microbiota 8 weeks after SCI [24]. The basic research discusses the relationship between gut microbiota and locomotor function and also observes the changes in intestinal function, immune function, and cytokines in relation to the gut microbiota. These animal model-based findings are of great significance to the clinical research on gut microbiota after SCI.

Complications after SCI and their association with gut microbiota (Fig. 1)

Intestinal dysfunction

Intestinal dysfunction, which is called neurogenic bowel dysfunction (NBD), often occurs after SCI. Constipation and fecal incontinence are the main manifestations of NBD; the reported prevalence rates of constipation and fecal incontinence in these patients are 40–58% and 2–61%,

respectively [15, 25-27]. After SCI, the autonomic nerve reflex becomes abnormal, resulting in poor colon compliance, loss of anal sphincter control, prolonged intestinal transit time, abdominal distention, dysdefecation, and other diseases [28]. After SCI, extensive damage of the internal enteric nervous system (ENS) occurs, which destroys the regulation of local reflexes associated with secretion of colonic contents, propulsion, and colon segmentation [29]. Neural damage destroys the intestinal transport function and limits the delivery of important nutrients to the distal colonic microbiota. Changes in mucin production destroy the mucus layer, on which colonization of intestinal microflora occurs, and cause gut dysbiosis to varying extents [30]. The GALT is also innervated by the sympathetic nerve of the spinal cord. Changes in the sympathetic nerve tone caused by SCI destroy the immune homeostasis of the GALT and affect the regulation of gut microbiota by mucosal immune system [31]. In addition, studies have shown that an increase in intestinal barrier permeability and the occurrence of enterogenous infections after SCI are also important causes of intestinal dysfunction [32]. Kigel et al. Showed that the probiotic intervention improved the intestinal immune function and the recovery of motor function in SCI mice [21]. Our previous study showed that the melatonin intervention improved the integrity of intestinal barrier and accelerated the intestinal transit time and motor function recovery in SCI mice [22]. It is not clear whether the improvement in the intestinal function after SCI is beneficial for locomotory recovery. It is important to develop a more appropriate intestinal management scheme for patients with SCI and also to provide data support for the development of appropriate evidence-based treatment strategies by studying the intestinal function and gut microbiota after SCI.

Anxiety and depression

In addition to motor and sensory injury, SCI is often accompanied by a high incidence rate of anxiety and depression, which reduces the quality of life and increases the risk of suicide [33-35]. These psychosocial conditions may be associated with post-traumatic depression and anxiety owing to drastic changes in life style, high medical costs, and complications, such as pain and autonomic nervous dysfunction. However, physiological changes that directly affect the emotional and mental health after SCI also exist. Recently, the gut-brain axis has been regarded as a powerful regulator of emotional and mental health. When specific types of bacteria colonize the gut, they can activate the signal network in the brain, thus triggering anxiety-like behaviors. Animal experiments show that vagal afferent nerve could be stimulated by fatty acids produced by the gut microbiota and that vagal nerve transection makes mice resistant to anxiety-like behaviors caused by microbiota [36, 37]. In SCI models of rats and mice, physiological changes occur that can directly affect emotional and mental health [38, 39]; Kurokawa et al. Showed that transplantation of fecal bacteria from healthy rats to SCI rats could reverse the gut dysbiosis induced by SCI and that it had potential anti-anxiety function [40]. However, the molecular mechanism of its therapeutic effect is not clear.

Metabolic diseases

SCI often causes limb disability; cervical SCI can cause quadriplegia. The metabolic function of paraplegia or quadriplegia patients is obviously damaged, and their body fat content is higher than that of healthy people [41, 42]. Patients with SCI also have high levels of intramuscular fat, which leads to impaired insulin resistance and glucose sensitivity [43, 44]. Changes in the composition of gut microbiota with regard to *Bacteroidetes* and *Firmicutes* (the two most prevalent bacterial taxa in mice and humans) may cause or lead to chronic metabolic disorders after SCI [45, 46]. Our

clinical research showed that the serum lipid profiles in peripheral blood are abnormal in patients with contusion cervical SCI; the abundance of *Faecalibacterium spp.*, *Megamonas spp.*, and *Prevotella spp.* in feces is related to lipid metabolism indices, and these species are considered as intervention targets of lipid metabolism abnormality after SCI. There was a positive correlation between gut microbiota (the abundance of *lactobacillus*) and the level of glucose in peripheral blood [47]. Our study shows that metabolic abnormalities in patients with SCI are related to changes in intestinal microecology, but the causal relationship between them is not clear. In addition, it is necessary to maintain a sedentary lifestyle for a long time after SCI, which is likely to directly or indirectly alter gut microbiota.

Neuropathic Pain

It has been reported that the incidence rate of neuropathic pain in patients with SCI, which usually develops into a chronic disease in the first year after SCI, is 40–50% [48, 49]. At present, its treatment is difficult, and the efficacy of the recommended treatment scheme is limited [50]. The pathological mechanism of neuropathic pain is not clear. Recent studies have shown that gut microbiota can regulate neuropathic pain [51, 52]. Gut microbiota can also stimulate neutrophils, monocytes, and secretory cells of the colon tissue in natural immune system to secrete biomolecules that inhibit pain [53]. Gut microbiota can synthesize and release neurotransmitters and short-chain fatty acids (SCFAs), regulate the metabolism of steroids and bile acids, and affect the release of neuropeptides and hormones by intestinal endocrine cells. These molecules can penetrate into the blood and/or lymphatic system, affect the neural information transmitted by the vagus nerve and spinal cord afferent neurons, and thus affect the pain response mediated by the central nervous system (CNS) and spinal cord. A recent study [54] emphasized the key role of gut

microbiota in the maturation, morphology, and immune function of central microglia. In view of the important role of microglia in the pathogenesis of pain, it may indirectly indicate that gut microbiota can affect the central pain process.

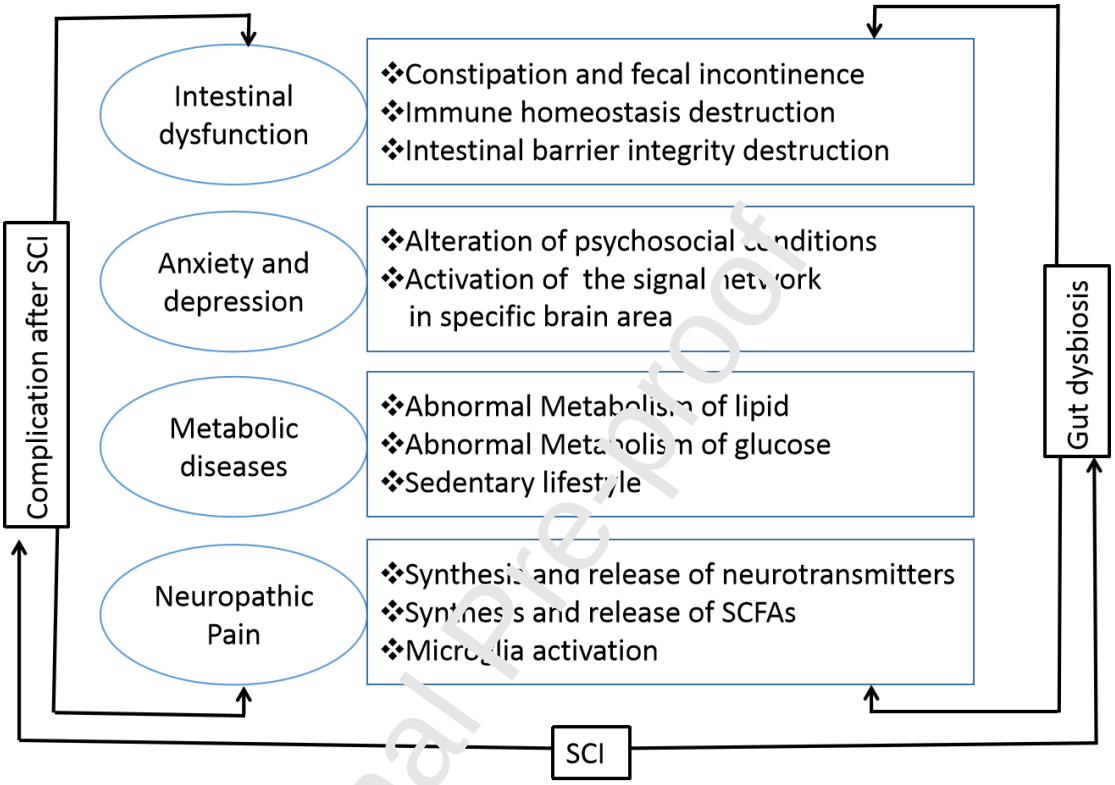

Fig.1. Gut dysbiosis after SCI could contribute to the various comorbidities typically attributed to intestinal function, mood, metabolism and pain. Various symptoms of comorbidities was related with gut microbiota. Gut dysbiosis has been implicated in the onset and progression of common postinjury comorbidities, which caused or exacerbated intraspinal pathology and neurological impairment.

People with SCI can be prone to complications such as pneumonia, septicemia, urinary tract infections, cardiac diseases, pressure ulcers, chronic pain, and colorectal, bladder and sexual dysfunction, which are frequently reported complications [55-62]. Most or all complications may occur together, which may increase the clinical severity of medical conditions. Surveys among the

SCI population showed that the recovery of bowel function was often prioritized above the ability to walk [63, 64]. Intestinal dysfunction results in the loss of the ability to live independently. The change in lifestyle and heavy economic burden may cause psychological disorders after SCI. Studies indicated that the prevalence of anxiety and depression among the SCI population was 33.7-58.3% [65, 66]. SCI also enhances the risk or frequency of metabolic disorders including obesity, diabetes, and liver dysfunction [67-70], which is often attributed to inactivity in people with SCI. Lipid metabolism disorder is one of the characteristics of metabolic disorder after SCI; it is reported that 76% of SCI patients will have at least one abnormal lipid parameter [71]. SCI-related neuropathic pain is one of the most common symptoms in the long lasting chronic phase and severely decreases the quality of life [72]. The incidence of these four complications in SCI was close to or more than 50%. This can result in physically and psychologically devastating trauma to persons with SCI. Gut dysbiosis creates a feed-forward cycle with complications, and gut microbiota may represent a potential solution.

Most of the commensal microbiota of the human body reside in the gut and the cell number is roughly the same compare to that of human cells [73]. However, microbiota contain ~100 fold more genes than the human genome [74]. This vast microbial network has profound biological effects on the host [75-77]. After SCI, gut dysbiosis can affect the occurrence of complications related to the intestinal function, neurological function, immune function, energy metabolism, and others. We speculate that the complications related to paralysis or life-related psychological stress after SCI may be related to gut microbiota. Future studies will continue to fill the gap in this field.

"Microbiota-gut-brain" axis

The gut-brain axis is an information exchange network connecting the gut and the brain. The gut "talks" to the brain via a variety of physiological and biochemical channels. The importance of the gut-brain axis in maintaining homeostasis has long been recognized. In the past two decades, microorganisms, as the key regulators of the gut-brain function, have attracted researchers' attention and have led to the concept of "microbiome-gut-brain axis." The microbiota communicates with the brain via the autonomic nervous system, immune system, tryptophan metabolism system, endocrine system, intestinal nervous system, and gut microbial metabolites [78, 79]. In the bidirectional regulation process, gut microbial metabolites play a very important role in the "microbiota-gut-brain" axis. They can not only regulate the CNS by directly stimulating the epithelium of the digestive tract, but also make a series of changes under the descending signal transduction of the CNS through the alteration of intestinal endocrine, motility and gut microbiota. "Microbiota-gut-brain axis" is receiving progressively increasing attention from researches in the field of psychiatry, neurodevelopment, and age-related physiological basis of CNS diseases.

Gut microbiota can produce neuroactive metabolites (such as SCFAs, branched fatty acids, and peptidoglycans) and neurotransmitters (such as γ-aminobutyric acid, 5-hydroxytryptamine, dopamine, acetylcholine) to activate neurons in the intestine and CNS [80]. Microbiota promote the production of serotonin in the intestinal tract and circulating blood of mice and affect anxiety, hyperactivity, and cognitive activity [32]. Microorganisms regulate the expression of 5-hydroxytryptamine receptor, NR2A, and brain-derived neurotrophic factor [81, 82] and affect the formation of cortical myelin sheath and the function of blood-brain barrier [83, 84]. Microbial metabolites, such as acetate, propionate, butyrate, etc., can enter the CNS to play critical roles by crossing the blood-brain barrier. Metabolites produced by *Roseburia sp.* and *Faecalibacterium sp.*

easily cross the blood-brain barrier [85]. These studies have shown that gut microbiota and its metabolites play an important role in regulating the CNS.

Recent data showed that gut microbiota play an important role in the occurrence and progression of CNS diseases (including autism, pain, depression, anxiety, stroke, Parkinson's disease) [86-90]. Under pathological conditions, the permeability of blood-brain barrier alters, and lipopolysaccharide (LPS), bacterial metabolites, and various inflammatory factors enter the CNS, promoting the occurrence of neuroinflammation and even CNS diseases [91]. For example, gut dysbiosis caused by an increase in *Methanobrevibacter* can occur in the intestine of patients with multiple sclerosis [92]. Moreover, animal experiments further showed that gut microbiota is the initial inflammatory mediator of demyelination in multiple sclerosis and other diseases [93]. The role of gut microbiota in the pathogenesis of Alzheimer's disease has also been preliminarily elucidated, that is, gut microbiota can release a large number of amyloid proteins and LPSs, which could regulate disease-related signal pathways and further produce proinflammatory factors related to Alzheimer's disease [94]. Patients with Parkinson’s disease (PD) display alterations in gut microbiota composition. Gut microbiota from PD mice induced impairment of motor function and decrease in striatal neurotransmitter levels in normal mice. Fecal bacterial transplantation (FMT) can play a neuroprotective role in PD mice by inhibiting neuroinflammation and reducing the activity of TLR4/TNF-α signal pathways [95]. The above study shows that gut dysbiosis is an important factor and mechanism involved in the occurrence and development of some CNS diseases.

The research on SCI from the perspective of intestinal microbiota is in its infancy. Modes of communication by which these bacteria likely affect the spinal cord structure and function are not

clear. Current research data show that gut microbiota can affect GALT immune cells and consequently, the synthesis of inflammatory and immunoregulatory cytokines in immune cells [21]. Our data suggest that the content of SCFAs in gut microbiota significantly decreased after SCI, which may be related to inflammation of the spinal cord and intestinal tissues after SCI (data not published). After SCI, the permeability of intestinal epithelial cells increased, and bacteria were translocated and implanted in peripheral blood and various organs [21]. Our previous studies have shown that the permeability of blood-spinal cord barrier increases after SCI [96]. It is not clear whether the gut microbiota entering the blood can directly affect the function of the spinal cord and brain through the injured blood-spinal cord barrier.

Research strategies targeting "microbiota-gut-brain axis" (Fig. 2)

Germ-free (GF) animals

Germ-free (GF) animals are animals that have not been exposed to microbiota since their birth. Animals lacking microbiota have extraordinarily different development and physiology than animals hosting commensal bacteria; in these animals, the immune system is damaged, hormonal signals are maladjusted, metabolism is altered, and neural transmission is different from that of traditional animals. Although there are many limitations mentiented, GF animals are still valuable tools for human beings to gain insights into the behavior, physiology, and neurobiology of the host [97]. They are important starting points to answer whether the microbiota or known strains participate in a specific physiological and pathological process. In recent years, GF animals have often been used to explain the pathogenesis of CNS diseases. When fecal samples obtained from patients with depression were transplanted into GF mice, the recipient mice showed anxiety and

depression like behaviors [98]. GF mice that received fecal microbial transplantation from patients with PD showed abnormal movement function and alteration of SCFA levels in the feces [90, 99]. The GF app/PS1 mouse model developed by Harach et al. showed that gut microbiota were related to the pathological deposition of β-amyloid plaques in those with Alzheimer's disease [100]. The above studies on GF animals explain the causal relationship of the gut microbiota with the occurrence and development of corresponding diseases and inspired the research on SCI. The disease phenotype appearing after the transplantation of abnormal microbiota associated with SCI to GF mice is worthy of attention. In subsequent studies, fecal samples should be collected from SCI patients (especially chronic SCI patients) and transplanted to GF mice to analyze the relationship between gut microbiota and complications occurring after SCI through the detection of serum metabolic spectrum and behavior.

Fecal microbiota transplantation (FMT)

Fecal microbiota transplantation (FMT), as a special "organ" transplantation, involves the separation of the functional flora from the feces of healthy people and its transplantation into the digestive tract of patients so as to achieve the treatment of the disease by reconstructing the gut microbiota of patients [101]. As an ancient medical technology, FMT has been recorded in traditional Chinese medicine 3000 years ago [102]. The mechanism of action of FMT on the intestinal tract may include the restoration of intestinal microenvironment, remodeling of the structure and function of the intestinal microecology, and affecting the signal exchange between microbiota and the host [103]. In addition to affecting the intestinal immune system, endocrine system, and ENS, FMT can also affects the CNS. In 2015, a clinical report showed that 65 year old male patients with acute SCI quadriplegia suffered from repeated *Clostridium difficile*

infection when antibiotics were used to treat pneumonia, and all kinds of antibiotics were ineffective. Doctors transplanted fecal bacteria from their healthy sons to the patients, and the pneumonia was cured. The infection did not recur during the 12-week follow-up period [104]. However, the authors in this study did not evaluate the motor function of the patient after FMT. Kurokawa et al. established anxiety models through incomplete unilateral cervical SCI and conducted them FMT intervention twice in succession; this treatment improved gut dysbiosis and inhibited the development of anxiety-like behavior [40]. We also tried to treat SCI mice by FMT intervention. The data show that FMT can improve the recovery of motor function, which may be related to the regulation of inflammation by SCFAs (data not published). The corresponding mechanism will be explored further. At present, hundreds of trials are being carried out to determining the efficacy of FMT in treating/managing a variety of disease including infectious disease, metabolic diseases, immune diseases, cancers, and neurological diseases [105]. If the clinical trial of the effect of FMT intervention on SCI is carried out in the future, standard donor selection and microbiological screening procedures need to be established, and the composition and structure of the donor community should be considered; additionally, the physical condition and receptivity of the recipient also should be comprehensively assessed, to reduce the risk of infection and death in the recipient.

Probiotics

The term probiotics was first put forward in 1965, which originated from the Greek word "good for life," and its definition is gradually supplemented and deepened with a deepening of people's understanding of it. Probiotics can regulate immune function, improve intestinal barrier function, generate organic acids and anti-microbial products, and interact with a host and its flora

[106]. Some strains are widely used in the field of medical care and alternative medicine as natural agents to regulate the balance of gut microbiota and to affect the brain health and mental health. The regulation of probiotics and "microbiota-gut-brain axis" is considered as a new therapeutic tool for intestinal dysfunction [107]. Research shows that the administration of *Lactobacillus reuteri* modulates neural-dependent motility reflexes by communicating with the brain in mice [108]; furthermore, *L. reuteri* has been shown to interact with the gut-brain axis in rats through the modulation of afferent sensory nerves that influence gut motility [109]. Kigerl et al. administered a probiotic-based (VSL# 3) intervention to SCI mouse models for 35 days, from the time of injury. The results showed that probiotics significantly reduced the volume of injury at its epicenter, which was conducive to the recovery normal behavior of mice [21]. However, it is not clear whether the neuroprotective effect of VSL#3 is related to its interaction with the "microbiota-gut-brain axis." Patients with SCI in a clinical setting often take probiotic (such as Bifico) to relieve gastrointestinal dysfunction although its neuroregulatory function is not clear. Time point of initial intervention and the cycle of intervention are both the key points worthy of further exploration. In the future, we can select specific strains with long-term clinical safety for research and further observe their colonization in the intestinal tract and their regulatory effect on the immune function and neural function, so as to provide new ideas and data support for the clinical treatment of SCI.

Bacteriophage

Bacteriophage are viruses that specifically infect bacteria and can precisely perturb specific bacteria within complex microbial communities. The ability of a phage to specifically infect selected bacteria is governed by intricate intermolecular interactions between bacterial cell surface

molecules and the host recognition domain of the phage [110, 111]. Phages participate in the dynamic interaction between bacteria and the host and regulate the human immune system [112]. The prospect of using bacteriophages for treatment emerged soon after its discovery in 1917. Indeed, at the beginning of the 20th century, phage therapy was introduced into clinical applications at various capacities, the most common being antimicrobial therapy. However, phage therapy soon fell out of favor after the advent of broad-spectrum antibiotics and research into the use of phages for therapeutic purposes has been at a standstill for decades [113, 114]. In recent years, with an increase of bacterial drug resistance, researchers have paid more attention to phages as substitutes for antibacterial drug [115, 116]. It is hoped that through the research of phage, the specific bacteria of intestinal microbiota can be targeted intervention to achieve the purpose of treating diseases.In addition, while studying phages, we should pay more attention to the possible adverse reactions caused by the high penetration of phages.

Brain imaging

Brain imaging technology can be used to study the communication among the brain, gut, and microbiota with regard to brain-gut interactions. The development of brain imaging technologies, such as positron emission tomography and nuclear magnetic resonance imaging (MRI), has deepened the understanding of the structure, function, and metabolic characteristics of brain regions and networks. Standardization of image acquisition and progress in computational methods have made studying large data sets of imaging, recognizing network attributes, and integrating them with non-imaging data possible. The ongoing work in the field of brain imaging includes linking gut microbial ecology to large-scale brain networks [117, 118]. This approach will help determine how microbiota affects brain function and possibly recognize multiple

mediators of the gut-brain axis. Fernandez Rea et al. evaluated the cognitive function of obese and non-obese individuals, detected brain microstructure by MRI and diffusion tensor imaging, and measured intestinal microflora composition using microbial sequencing. The results showed that the composition of gut microbiota of obese and non-obese subjects were different. In particular, the richness of *Actinobacteria* was highly related to the scores of cognitive tests and the changes in neural activities in the thalamus, hypothalamus, and amygdala. This supports that obesity affects the composition of gut microbial population and subsequent cognitive performance [119]. By using a machine learning classifier to quantitatively evaluate the association intensity of the brain area and microbiome, it was found that the change in brain structure is related to the change in the diet-dependent gut microbiome [120]. These studies provide new ideas for the investigations of correlation between brain area, microorganism and disease. Using magnetic resonance technology, it was found that the brain area which plays an important role in the processing of information and emotion has changed in patients with SCI [121, 122]. Yun Guo et al. demonstrated white matter microstructure alterations in patients with spinal cord injury as assessed by diffusion tensor imaging [123]. Additionally, they provided functional magnetic resonance imaging evidence for abnormal changes in brain function brought about by the loss of physical movement and sensory feedback in the lower limbs in chronic SCI [124]. But the existing research did not correlate the changes of gut microbiota and the brain area. Integration of multimodal brain imaging and big data of multi-omics help understand the changes of brain structure and function associated with the disturbance of microbiota-gut-brain interaction and their biological significance in the pathophysiology and intervention after SCI.

The above research strategies are very valuable because they facilitate a better understanding

of the "microbiota-gut-brain axis." With these research strategies, we can further study the exact relationship of the occurrence of SCI with development of secondary injury and complications after SCI and gut microbiota, clarify the mode of interaction of microbiota and/or metabolites with the host, and pave the way for clinical transformation.

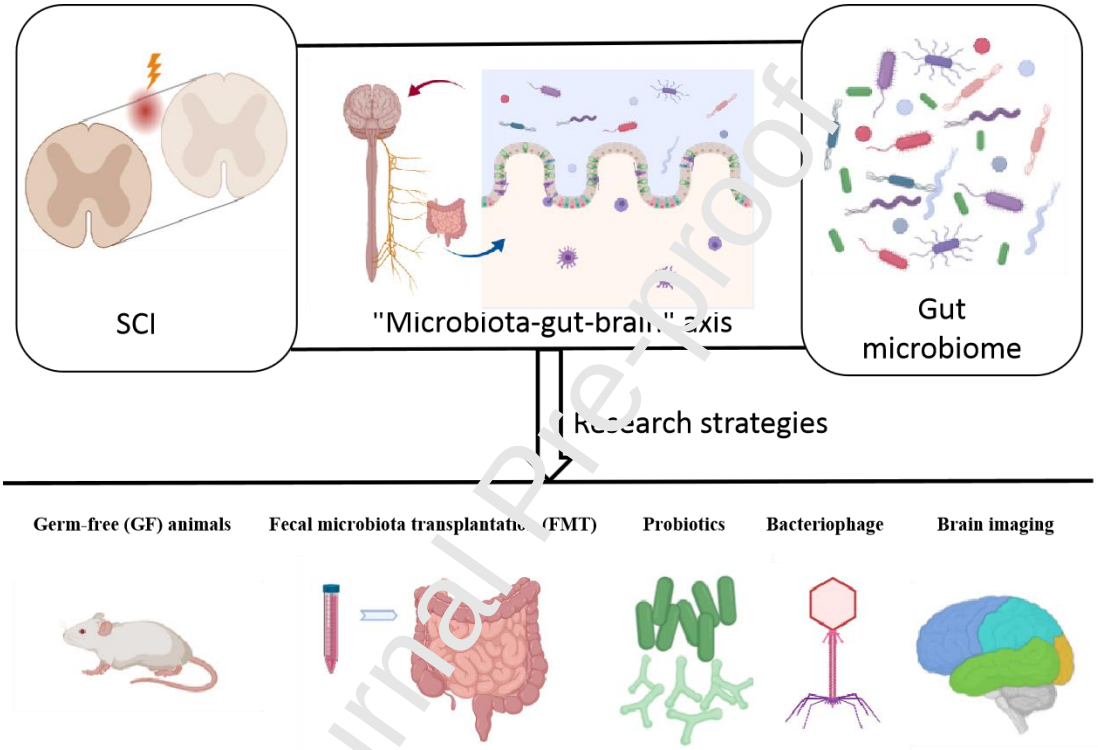

Fig. 2."Microbiota-gut-brain" axis - based research strategies. Gut microbiota “talk” to CNS via the autonomic nervous system, immune system, tryptophan metabolism system, endocrine system, intestinal nervous system, and gut microbial metabolites. Recent research has elucidated gut microbiome interventions for promoting human health and for combating disease. These research strategies included GF animals, FMT, probiotics, bacteriophage and brain imaging. Collectively, these research strategies, when used alone or in combinations, will explore the host - microbiome modes of interaction.

Challenges and prospects of SCI and gut microbiota

The last decade has witnessed a remarkable leap in microbiome research. In the early stages, descriptive research on detailed characterization of microbiome alterations in healthy and diseased states was focused on; at present, growing research exploring the causal relationship between microbial composition and various phenotypes is being conducted [125, 126]. Significant progress in microbiological research is conducive to the interpretation and even prediction of human health and disease status by microbiological and other physiological and biochemical indicators, as well as the adoption of personalized interventions. Because of the complexity of gut microbiota, when using experimental animals to study gut microbiota, not only genetic background, age, feeding environment, diet, and other factors that can highly reshape the intestinal microflora need to be considered, but also the differences between experimental animals and humans, including microbiological composition, diet, immunology, complications, and social factors, also should be considered. Because of the differences of geography, race, and nutritional background, human beings tend to show more heterogeneous microbial groups and phenotypes than do animal models. In multi-center clinical microbial studies, individual differences should be considered; in addition, unified standards for sample collection, storage, sub-packaging, numbering and, other processing should be established. Subsequently, data should be unified, analyzed, and interpreted. When using live microbiota (such as FMT or probiotics) for intervention, it is necessary to consider the transmission of pathogenic factors and other safety risks while considering their potential therapeutic value for the diseases.

Bacterial extracellular vesicles (EVs) are bilayered lipid membrane structures with a diameter ranging from 20 to 400 nm [127]. Emerging evidence has shown that bacterial EVs are implicated

in human disease physiopathology and seem to modulate the occurrence and severity of diseases in some cases [128-130]. In microorganisms, EVs carry several types of molecules: proteins, glycoproteins, mRNAs, and micro RNAs. As such, they could affect the functioning of cells that uptake them. Interactions between bacterial EVs and the host act as potent immune modulator agents [131]. Studying EVs opens up a new field of possibilities to better understand the interplay between host and bacteria, though whether microbiota-derived EVs could potentially become a clinical target for innovative therapies will need to be further evaluated.

Conclusions

Contusion SCI is a kind of neural injury disease which is different from primary neural injury disease, there is no causal relationship between gut microbiota and SCI. However, there is a correlation or possible causal relationship between gut microbiota and secondary injury/complications following injury. Researchers need to study the post-SCI changes in gut microbiota in multiple dimensions, such as damage degree, damage time, damage segment, etc., and further verify the results using GF mice and microbial transplantation. In addition, most of the microbial community research relies on 16S RNA and metagenome sequencing technology and it is difficult to identify microbiota at the level of strains and to carry out targeted precise intervention. However, with the progress of sequencing technology and the optimization of data processing, these problems may be solved. Future research may further explain the pathophysiological mechanism of association between SCI and gut microbiota with the help of new technologies, sort out the mode of interaction between gut microbiota and host, and help develop personalized targeted bacterial therapies and drugs based on microbiota or its metabolites.

Abbreviations

CNS, Central nervous system; ENS, Enteric nervous system; FMT, Fecal microbiota transplantation; GALT, Gut associated lymphoid tissue; GF, Germ-free; MRI, Magnetic resonance imaging; NBD, Neurogenic bowel dysfunction; PD, Parkinson's disease; SCFA, Short-chain fatty acids; SCI, Spinal cord injury; LPS, lipopolysaccharide.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgments

The study was supported by research grant from National Natural Science Foundation of China (Grant no. 81901272).

References

[1] Bradbury EJ, Burnside ER. Moving beyond the glial scar for spinal cord repair. Nat Commun. 2019;10(1):3879.

[2] Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Traumatic Spinal Cord Injury: An Overview of Pathophysiology, Models and Acute Injury Mechanisms. Front Neurol. 2019;10:282.

[3] Ramer LM, Ramer MS, Bradbury EJ. Restoring function after spinal cord injury: towards clinical translation of experimental strategies. Lancet Neurol. 2014;13(12):1241-56.

[4] Sofroniew MV. Dissecting spinal cord regeneration. Nature. 2018;557(7705):343-50.

[5] Squair JW, DeVeau KM, Harman KA, Poormasjedi-Meibod MS, Hayes B, Liu J, et al. Spinal Cord Injury Causes Systolic Dysfunction and Cardiomyocyte Atrophy. J Neurotrauma. 2018;35(3):424-34.

[6] Hsam NBO, Angstwurm K, Peters S, Fuchs K, Schuierer G, Bogdahn U, et al. Fulminant Acute Ascending Hemorrhagic Myelitis Treated with Eculizumab. Front Neurol. 2017;8:345.

[7] White AR, Holmes GM. Anatomical and Functional Changes to the Colonic Neuromuscular Compartment after Experimental Spinal Cord Injury. J Neurotrauma. 2018;35(9):1079-90.

[8] Ebert E. Gastrointestinal involvement in spinal cord injury: a clinical perspective. J Gastrointestin Liver Dis. 2012;21(1):75-82.

[9] Simpson LA, Eng JJ, Hsieh JTC, Wolfe DL, Evidenc SCIR. The Health and Life Priorities of Individuals with Spinal Cord Injury: A Systematic Review. J Neurotrauma. 2012;29(8):1548-55.

[10] Anderson KD. Targeting recovery: Priorities of the spinal cord-injured population. J Neurotrauma. 2004;21(10):1371-83.

[11] Middleton JW, Dayton A, Walsh J, Rutkowski SB, Leong G, Duong S. Life expectancy after spinal cord injury: a 50-year study. Spinal Cord. 2012;50(11):803-11.

[12] Sezer N, Akkus S, Ugurlu FG. Chronic complications of spinal cord injury. World J

Orthop. 2015;6(1):24-33.

[13] Pan Y, Liu B, Li R, Zhang Z, Lu L. Bowel dysfunction in spinal cord injury: current perspectives. Cell Biochem Biophys. 2014;69(3):385-8.

[14] Ahuja CS, Wilson JR, Nori S, Kotter MRN, Druschel C, Curt A, et al. Traumatic spinal cord injury. Nat Rev Dis Primers. 2017;3:17018.

[15] Tate DG, Forchheimer M, Rodriguez G, Chiodo A, Cameron AP, Meade M, et al. Risk Factors Associated With Neurogenic Bowel Complications and Dysfunction in Spinal Cord Injury. Arch Phys Med Rehabil. 2016;97(10):1670-86.

[16] Cervi AL, Lukewich MK, Lomax AE. Neural regulation of gastrointestinal inflammation: Role of the sympathetic nervous system. Auton Neurosci. 2014;182:83-8.

[17] Gungor B, Adiguzel E, Gursel I, Yilmaz B, Gursel M. Intestinal Microbiota in Patients with Spinal Cord Injury. PLoS One. 2016;11(1).

[18] Zhang C, Zhang W, Zhang J, Jing Y, Yang M, Du L, et al. Gut microbiota dysbiosis in male patients with chronic traumatic complete spinal cord injury. J Transl Med. 2018;16(1):353.

[19] Ghosh TS, Das M, Jeffery IB, O'Toole PW. Adjusting for age improves identification of gut microbiome alterations in multiple diseases. Elife. 2020;9.

[20] Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019;99(4):1877-2013.

[21] Kigerl KA, Hall JCE, Wang LL, Mo XK, Yu ZT, Popovich PG. Gut dysbiosis impairs

recovery after spinal cord injury. J Exp Med. 2016;213(12):2603-20.

[22] Jing YL, Yang DG, Bai F, Zhang C, Qin C, Li D, et al. Melatonin Treatment Alleviates Spinal Cord Injury-Induced Gut Dysbiosis in Mice. J Neurotrauma. 2019;36(18):2646-64.

[23] Shavelle RM, DeVivo MJ, Brooks JC, Strauss DJ, Paculdo DR. Improvements in long-term survival after spinal cord injury? Arch Phys Med Rehabil. 2015;96(4):645-51.

[24] O'Connor G, Jeffrey E, Madorma D, Marcillo A, Abreu MT. Deo SK, et al. Investigation of Microbiota Alterations and Intestinal Inflammation Post-Spinal Cord Injury in Rat Model. J Neurotrauma. 2018;35(18):2159-66.

[25] Koo BI, Bang TS, Kim SY, Ko SH, Kim W, Ko HY. Anorectal Manometric and Urodynamic Parameters According to the Spinal Cord Injury Lesion. Ann Rehabil Med. 2016;40(3):528-33.

[26] Ozisler Z, Koklu K, Ozel S, Unsal-Delialioglu S. Outcomes of bowel program in spinal cord injury patients with neurogenic bowel dysfunction. Neural Regen Res. 2015;10(7):1153-8.

[27] Longo WE, Ballantyne GH, Modlin IM. The colon, anorectum, and spinal cord patient. A review of the functional alterations of the denervated hindgut. Dis Colon Rectum. 1989;32(3):261-7.

[28] Faaborg PM, Christensen P, Rosenkilde M, Laurberg S, Krogh K. Do gastrointestinal transit times and colonic dimensions change with time since spinal cord injury? Spinal Cord. 2011;49(4):549-53.

[29] Furness JB, Callaghan BP, Rivera LR, Cho HJ. The enteric nervous system and gastrointestinal innervation: integrated local and central control. Adv Exp Med Biol. 2014;817:39-71.

[30] Kigerl KA, Zane K, Adams K, Sullivan MB, Popovich PG. The spinal cord-gut-immune axis as a master regulator of health and neurological function after spinal cord injury. Exp Neurol. 2020;323:113085.

[31] Straub RH, Wiest R, Strauch UG, Harle P, Scholmerich J. The role of sympathetic nervous system in intestinal inflammation. Gut. 2006;55(11):1640-9.

[32] Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell. 2015;161(2):264-76.

[33] Lim SW, Shiue YL, Ho CH, Yeh SC, Kao PH, Wang JJ, et al. Anxiety and Depression in Patients with Traumatic Spinal Cord Injury: A Nationwide Population-Based Cohort Study. PLoS One. 2017;12(1):e0169623.

[34] Lim SW, Eric Niyam TT, Ho CH, Shiue YL, Wang JJ, Chio CC, et al. Increased Risk of Anxiety or Depression After Traumatic Spinal Cord Injury in Patients with Preexisting Hyperlipidemia: A Population-Based Study. World Neurosurg. 2017;106:402-8.

[35] Kennedy P, Rogers BA. Anxiety and depression after spinal cord injury: a longitudinal analysis. Arch Phys Med Rehabil. 2000;81(7):932-7.

[36] Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor

expression in a mouse via the vagus nerve. Proc Natl Acad Sci U S A. 2011;108(38):16050-5.

[37] Lal S, Kirkup AJ, Brunsden AM, Thompson DG, Grundy D. Vagal afferent responses to fatty acids of different chain length in the rat. Am J Physiol Gastrointest Liver Physiol. 2001;281(4):G907-15.

[38] Luedtke K, Bouchard SM, Woller SA, Funk MK, Aceves M, Hook MA. Assessment of depression in a rodent model of spinal cord injury. J Neurotrauma. 2014;31(12):1107-21.

[39] Craig A, Guest R, Tran Y, Middleton J. Cognitive Impairment and Mood States after Spinal Cord Injury. J Neurotrauma. 2017;34(6):1156-63.

[40] Schmidt EKA, Torres-Espin A, Raposo PJF, Madsen KL, Kigerl KA, Popovich PG, et al. Fecal transplant prevents gut dysbiosis and anxiety-like behaviour after spinal cord injury in rats. PLoS One. 2020;15(1):e0226128.

[41] Gorgey AS, Mather RL, Poarch HJ, Gater DR. Influence of motor complete spinal cord injury on visceral and subcutaneous adipose tissue measured by multi-axial magnetic resonance imaging. J Spinal Cord Med. 2011;34(1):99-109.

[42] Gorgey AS, Dolbow DR, Dolbow JD, Khalil RK, Castillo C, Gater DR. Effects of spinal cord injury on body composition and metabolic profile - Part I. J Spinal Cord Med.2014;37(6):693-702.

[43] Boettcher M, Machann J, Stefan N, Thame C, Haring HU, Claussen CD, et al. Intermuscular Adipose Tissue (IMAT): Association With Other Adipose Tissue

Compartments and Insulin Sensitivity. J Magn Reson Imaging. 2009;29(6):1340-5.

[44] Elder CP, Apple DF, Bickel CS, Meyer RA, Dudley GA. Intramuscular fat and glucose tolerance after spinal cord injury - a cross-sectional study. Spinal Cord. 2004;42(12):711-6.

[45] Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. J Clin Invest. 2011;121(6):2126-32.

[46] Baothman OA, Zamzami MA, Taher I, Abubaker J, Abu-Farha M. The role of Gut Microbiota in the development of obesity and Diabetes. Lipids Health Dis. 2016;15.

[47] Zhang C, Jing YL, Zhang WH, Zhang J, Yang ML, Du LJ, et al. Dysbiosis of gut microbiota is associated with serum lipid profiles in male patients with chronic traumatic cervical spinal cord injury. Am J Transl Res. 2019;11(8):4817-4834. eCollection 2019

[48] Siddall PJ, McClelland JM, Rutkowski SB, Cousins MJ. A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. Pain. 2003;103(3):249-57.

[49] Werhagen L, Budh CN, Hultling C, Molander C. Neuropathic pain after traumatic spinal cord injury--relations to gender, spinal level, completeness, and age at the time of injury. Spinal Cord. 2004;42(12):665-73.

[50] Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet Neurol. 2015;14(2):162-73.

[51] Yang C, Fang X, Zhan GF, Huang NN, Li S, Bi JJ, et al. Key role of gut microbiota in anhedonia-like phenotype in rodents with neuropathic pain. Transl Psychiatry.2019;9.

[52] Guo R, Chen LH, Xing C, Liu T. Pain regulation by gut microbiota: molecular mechanisms and therapeutic potential. Br J Anaesth. 2019;123(5):637-54.

[53] Moloney RD, Johnson AC, O'Mahony SM, Dinan TG, Greenwood-Van Meerveld B, Cryan JF. Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. CNS Neurosci Ther. 2016;22(2):102-17.

[54] Erny D, Hrabe de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18(7):965-77.

[55] Shavelle RM, DeVivo MJ, Strauss DJ, Paculdo DR, Lammertse DP, Day SM. Long-term survival of persons ventilator dependent after spinal cord injury. J Spinal Cord Med. 2006;29(5):511-9.

[56] Sipski ML, Richards JS. Spinal cord injury rehabilitation: state of the science. Am J Phys Med Rehabil. 2006;85(4):310-42.

[57] Krassioukov A. Autonomic function following cervical spinal cord injury. Respir Physiol Neurobiol. 2009;169(2):157-64.

[58] Stampas A, Dominick E, Zhu L. Evaluation of functional outcomes in traumatic spinal cord injury with rehabilitation-acquired urinary tract infections: A retrospective study. J Spinal Cord Med. 2019;42(5):579-85.

[59] Dijkers M, Bryce T, Zanca J. Prevalence of chronic pain after traumatic spinal cord injury: a systematic review. J Rehabil Res Dev. 2009;46(1):13-29.

[60] Moza R, Dimaio JM, Melendez J. Deep-tissue dynamic monitoring of decubitus ulcers: wound care and assessment. IEEE Eng Med Biol Mag. 2010;29(2):71-7.

[61] Belanger LMA, Umedaly HS, Noonan VK, Park SE, Prince J, Thorogood NP, et al. Evaluation of a Clinical Protocol to Assess and Diagnose Neuropathic Pain During Acute Hospital Admission: Results From Traumatic Spinal Cord Injury. Clin J Pain. 2018;34(2):104-12.

[62] Krassioukov A, Elliott S. Neural Control and Physiology of Sexual Function: Effect of Spinal Cord Injury. Top Spinal Cord Inj Rehabil. 2017;23(1):1-10.

[63] Anderson KD. Targeting recovery: priorities of the spinal cord-injured population. J Neurotrauma. 2004;21(10):1371-83.

[64] Simpson LA, Eng JJ, Hsieh JT, Wolfe DL, Spinal Cord Injury Rehabilitation Evidence Scire Research T. The health and life priorities of individuals with spinal cord injury: a systematic review. J Neurotrauma. 2012;29(8):1548-55.

[65] Le J, Dorstyn D. Anxiety prevalence following spinal cord injury: a meta-analysis. Spinal Cord. 2016;54(8):626.

[66] Williams R, Murray A. Prevalence of depression after spinal cord injury: a meta-analysis. Arch Phys Med Rehabil. 2015;96(1):133-40.

[67] Cragg JJ, Noonan VK, Dvorak M, Krassioukov A, Mancini GB, Borisoff JF. Spinal cord

injury and type 2 diabetes: results from a population health survey. Neurology. 2013;81(21):1864-8.

[68] Gater DR, Jr. Obesity after spinal cord injury. Phys Med Rehabil Clin N Am. 2007;18(2):333-51, vii.

[69] Bauman WA, Spungen AM. Metabolic changes in persons after spinal cord injury. Phys Med Rehabil Clin N Am. 2000;11(1):109-40.

[70] Gorgey AS, Mather KJ, Gater DR. Central adiposity associations to carbohydrate and lipid metabolism in individuals with complete motor spinal cord injury. Metabolism. 2011;60(6):843-51.

[71] Vichiansiri R, Saengsuwan J, Manimmanakorn N, Patpiya S, Preeda A, Samerduen K, et al. The prevalence of dyslipidemia in patients with spinal cord lesion in Thailand. Cholesterol. 2012;2012:847462.

[72] Bryce TN, Biering-Sorensen F, Finnerup NB, Cardenas DD, Defrin R, Lundeberg T, et al. International spinal cord injury pain classification: part I. Background and description. March 6-7, 2009. Spinal Cord. 2012;50(6):413-7.

[73] Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016;14(8):e1002533.

[74] Belkaid Y, Naik S. Compartmentalized and systemic control of tissue immunity by commensals. Nat Immunol. 2013;14(7):646-53.

[75] Hooper LV, Littman DR, Macpherson AJ. Interactions Between the Microbiota and the

Immune System. Science. 2012;336(6086):1268-73.

[76] Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-Gut Microbiota Metabolic Interactions. Science. 2012;336(6086):1262-7.

[77] Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009;9(5):313-23.

[78] Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The Central Nervous System and the Gut Microbiome. Cell. 2016;167(4):915-32.

[79] Schroeder BO, Backhed F. Signals from the gut microbiota to distant organs in physiology and disease. Nat Med. 2016;22(10):1079-89.

[80] Tillisch K. The effects of gut microbiota on CNS function in humans. Gut Microbes. 2014;5(3):404-10.

[81] Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011;141(2):599-609, e1-3.

[82] Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A. 2011;108(7):3047-52.

[83] Hoban AE, Stilling RM, Ryan FJ, Shanahan F, Dinan TG, Claesson MJ, et al. Regulation of prefrontal cortex myelination by the microbiota. Transl Psychiatry. 2016;6:e774.

[84] Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, et al. The gut

microbiota influences blood-brain barrier permeability in mice. Sci Transl Med. 2014;6(263):263ra158.

[85] Byrne CS, Chambers ES, Morrison DJ, Frost G. The role of short chain fatty acids in appetite regulation and energy homeostasis. Int J Obes (Lond). 2015;39(9):1331-8.

[86] Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome. 2017;5.

[87] Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, et al. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. Nat Med. 2007;13(1):35-7.

[88] Foster J, Neufeld KA. Gut-brain axis: How the microbiome influences anxiety and depression. Trends Neurosci. 2013;36(5):305-12.

[89] Benakis C, Brea D, Caballero S, Faraco G, Moore J, Murphy M, et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal gamma delta T cells. Nat Med. 2016;22(5):516-23.

[90] Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell. 2016;167(6):1469-1480.e12..

[91] Cani PD, Bibiloni R, Knauf C, Neyrinck AM, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in

high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008;57(6):1470-81.

[92] Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, et al. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun. 2016;7.

[93] Joscelyn J, Kasper LH. Digesting the emerging role for the gut microbiome in central nervous system demyelination. Mult Scler. 2014;20(12):1553-9.

[94] Pistollato F, Cano SS, Elio I, Vergara MM, Giampieri F, Battino M. Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease.Nutr Rev. 2016;74(10):624-34.

[95] Sun MF, Zhu YL, Zhou ZL, Jia XB, Xu YD, Yang Q, et al. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: Gut microbiota, glial reaction and TLR4/TNF-alpha signaling pathway. Brain Behav Immun. 2018;70:48-60.

[96] Jing YL, Bai F, Chen H, Dong H. Melatonin prevents blood vessel loss and neurological impairment induced by spinal cord injury in rats. J Spinal Cord Med. 2017;40(2):222-9.

[97] Weger BD, Gobet C, Yeung J, Martin E, Jimenez S, Betrisey B, et al. The Mouse Microbiome Is Required for Sex-Specific Diurnal Rhythms of Gene Expression and Metabolism. Cell Metab. 2019;29(2):362-382.e8.

[98] Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol Psychiatry. 2016;21(6):786-96.

[99] Erny D, Prinz M. Microbiology: Gut microbes augment neurodegeneration. Nature. 2017;544(7650):304-5.

[100] Harach T, Marungruang N, Duthilleul N, Cheatham V, Mc Coy KD, Frisoni G, et al. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota (vol 7, 41802, 2017). Sci Rep. 2017;7.

[101] Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol. 2012;9(2):88-96.

[102] Faecal quality control. Nat Microbiol. 2019;4(8):1243.

[103] Liu SX, Li YH, Dai WK, Li XS, Qiu CZ, Kuan ML, et al. Fecal microbiota transplantation induces remission of infantile allergic colitis through gut microbiota re-establishment. World J Gastroenterol. 2017;23(48):8570-81.

[104] Brechmann T, Swol J, Krop Hammad V, Willert J, Aach M, Cruciger O, et al. Complicated fecal microbiota transplantation in a tetraplegic patient with severe Clostridium difficile infection. World J Gastroenterol. 2015;21(12):3736-40.

[105] Blaser MJ. Fecal Microbiota Transplantation for Dysbiosis - Predictable Risks. N Engl J Med. 2019;381(21):2064-6.

[106] Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat Rev Gastroenterol Hepatol. 2019;16(10):605-16.

[107] Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, et al. Consumption of

Fermented Milk Product With Probiotic Modulates Brain Activity. Gastroenterology. 2013;144(7):1394-U136.

[108] Wang BX, Mao YK, Diorio C, Pasyk M, Wu RY, Bienenstock J, et al. Luminal administration ex vivo of a live Lactobacillus species moderates mouse jejunal motility within minutes. FASEB J. 2010;24(10):4078-88.

[109] Kunze WA, Mao YK, Wang BX, Huizinga JD, Ma XL, Forsythe P, et al. Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening.J Cell Mol Med. 2009;13(8B):2261-70.

[110] Lemire S, Yehl KM, Lu TK. Phage-Based Applications in Synthetic Biology. Annu Rev Virol. 2018;5(1):453-76.

[111] Bertozzi Silva J, Storms Z, Sauvageau D. Host receptors for bacteriophage adsorption. FEMS Microbiol Lett. 2016;363(4).

[112] Huh H, Wong S, Jean JS, Slavcev R. Bacteriophage interactions with mammalian tissue: Therapeutic applications. Adv Drug Deliv Rev. 2019;145:4-17.

[113] Summers WC. The strange history of phage therapy. Bacteriophage. 2012;2(2):130-3.

[114] Voorhees PJ, Cruz-Teran C, Edelstein J, Lai SK. Challenges & opportunities for phage-based in situ microbiome engineering in the gut. J Control Release. 2020;326:106-19.

[115] Pires DP, Cleto S, Sillankorva S, Azeredo J, Lu TK. Genetically Engineered Phages: a Review of Advances over the Last Decade. Microbiol Mol Biol Rev. 2016;80(3):523-43.

.[116] Sabino J, Hirten RP, Colombel JF. Review article: bacteriophages in gastroenterology-from biology to clinical applications. Aliment Pharmacol Ther. 2020;51(1):53-63.

[117] Saulnier DM, Riehle K, Mistretta TA, Diaz MA, Mandal D, Raza S, et al. Gastrointestinal Microbiome Signatures of Pediatric Patients With Irritable Bowel Syndrome. Gastroenterology. 2011;141(5):1782-91.

[118] Iimia A, Van Horn JD. The structural, connectomic and network covariance of the human brain. Neuroimage. 2013;66:489-99.

[119] Fernandez-Real JM, Serino M, Blasco G, Puig J, Daunis-i-Estadella J, Ricart W, et al. Gut Microbiota Interacts With Brain Microstructure and Function. J Clin Endocrinol Metab. 2015;100(12):4505-13.

[120] Ong IM, Gonzalez JG, McIlwain SJ, Sawin EA, Schoen AJ, Adluru N, et al. Gut microbiome populations are associated with structure-specific changes in white matter architecture. Transl Psychiatry. 2018;8(1):6.

[121] Hawasli AH, Rudin J, Roland JL, Murphy RKJ, Song SK, Leuthardt EC, et al. Spinal Cord Injury Disrupts Resting-State Networks in the Human Brain. J Neurotrauma. 2018;35(6):864-73.

[122] Nicotra A, Critchley HD, Mathias CJ, Dolan RJ. Emotional and autonomic consequences of spinal cord injury explored using functional brain imaging. Brain. 2006;129:718-28.

[123] Guo Y, Gao F, Liu Y, Guo H, Yu W, Chen Z, et al. White Matter Microstructure

Alterations in Patients With Spinal Cord Injury Assessed by Diffusion Tensor Imaging. Front Hum Neurosci. 2019;13:11.

[124] Gao F, Guo Y, Chu H, Yu W, Chen Z, Chen L, et al. Lower-Limb Sensorimotor Deprivation-Related Brain Activation in Patients With Chronic Complete Spinal Cord Injury. Front Neurol. 2020;11:555733.

[125] Zmora N, Soffer E, Elinav E. Transforming medicine with the microbiome. Sci Transl Med. 2019;11(477).

[126] Hanage WP. Microbiology: Microbiome science needs a healthy dose of scepticism. Nature. 2014;512(7514):247-8.

[127] Dagnelie MA, Corvec S, Khammari A, Dreno B. Bacterial extracellular vesicles: A new way to decipher host-microbiota communications in inflammatory dermatoses. Exp Dermatol. 2020;29(1):22-8.

[128] Sang SG, Rong H, Wang CB, Xie YQ. Effects of Porphyromonas gingivalis extracellular vesicles on human periodontal ligament fibroblasts. Int J Clin Exp Med. 2014;7(2):379-83.

[129] Kang CS, Ban M, Choi EJ, Moon HG, Jeon JS, Kim DK, et al. Extracellular vesicles derived from gut microbiota, especially Akkermansia muciniphila, protect the progression of dextran sulfate sodium-induced colitis. PLoS One. 2013;8(10):e76520.

[130] Choi HI, Choi JP, Seo J, Kim BJ, Rho M, Han JK, et al. Helicobacter pylori-derived extracellular vesicles increased in the gastric juices of gastric adenocarcinoma patients

and induced inflammation mainly via specific targeting of gastric epithelial cells. Exp Mol Med. 2017;49(5):e330.

[131] Wu J, An M, Zhu J, Tan Z, Chen GY, Stidham RW, et al. A Method for Isolation and Proteomic Analysis of Outer Membrane Vesicles from Fecal Samples by LC-MS/MS. J Proteomics Bioinform. 2019;12(2):38-42.

Graphical abstract