NEUROSCIENCE

RESEARCH ARTICLE

S. De Santis et al. / Neuroscience xxx (2018) xxx–xxx

Radiomicrobiomics: Advancing Along the Gut-brain Axis Through Big Data Analysis

Silvia De Santis, [a] **David Moratal** [b] **and Santiago Canals** [a]*

[a] *Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas, Universidad Miguel Hernández, Sant Joan d'Alacant 03550, Spain*

[b] *Center for Biomaterials and Tissue Engineering, Universitat Politècnica de València, Valencia, Spain*

Abstract—The gut-brain axis communicates the brain with the gut microbiota, a bidirectional conduit that has received increasing attention in recent years thanks to its emerging role in brain development and function. Alterations in microbiota composition have been associated to neurological and psychiatric disorders, and several studies suggest that the immune system plays a fundamental role in the gut-brain interaction. Recent advances in brain imaging and in microbiome sequencing have generated a large amount of information, yet the data from both these sources need to be combined efficiently to extract biological meaning, and any diagnostic and/or prognostic benefit from these tools. In addition, the causal nature of the gut-brain interaction remains to be fully established, and preclinical findings translated to humans. In this ''Perspective" article, we discuss recent efforts to combine data on the gut microbiota with the features that can be obtained from the conversion of brain images into mineable data. The subsequent analysis of these data for diagnostic and prognostic purposes is an approach we call *radiomicrobiomics* and it holds tremendous potential to enhance our understanding of this fascinating connection.

This article is part of a Special Issue entitled: MRI & Neuroinflammation.

Key words: advanced MRI, microbiota, machine learning, big data, gut-brain axis, radiomicrobiomics.

INTRODUCTION: THE GUT BRAIN AXIS AND ITS INFLUENCE ON THE BRAIN

The term gut-brain axis refers to the bidirectional conduit that communicates the brain with the gut, two complex systems made up of thousands of millions of cells: neurons or bacteria. The communication between these organs involves immune (El Aidy et al., 2014), endocrine and neural pathways (Dinan and Cryan, 2017a). Gut-brain interactions have received increasing attention in recent years, as numerous findings suggest a fundamental influence of the gut microbiota on brain development and function. Some structures as the hypothalamus receiving visceral messages from the gut and with a critical role on stress regulation are of major importance in this tandem (Dinan and Cryan, 2012). Alterations in microbiota composition or dysbiosis have been already associated to neurological and psychiatric disorders, such as autism, depression and hyperphagic behaviors (Dinan and Cryan, 2017b).

Although most evidence has come from preclinical studies, important results show that ''humanizing" rodent microbiota through fecal transplants from patients also transfers some pathological features of the disease to the recipient animal. For instance, the transplantation of microbiota from major depression patients to microbiota-depleted rats, yet not that from age- and sex-matched controls, provoked physiological and behavioral features of depression in the transplanted animals (Kelly et al., 2016). Several studies suggest that the immune system plays a pivotal role in the gut-brain interaction (Kamada et al., 2013) and this association has been explored in autoimmune diseases, like multiple sclerosis. Remarkably, some of the bacterial species reduced in the gut of multiple sclerosis patients are also known to drive the production of immunosuppressive regulatory T cells (Ochoa-Repáraz and Kasper, 2017), a characteristic disease landmark. Despite these exciting findings, establishing a direct causal relationship for gut-brain interactions remains elusive in current neuropathological research (Zhao, 2013). As a result, preclinical findings have yet to be translated to humans. In this ''Perspective" article, we propose that combining advanced imaging techniques with microbiome analysis could dramatically speed up progress in these directions.

*Corresponding author. Address: Instituto de Neurociencias, Avenida Santiago Ramon y Cajal s/n, 03550 Sant Joan d'Alacant, Alicante, Spain.
E-mail address: scanals@umh.es (S. Canals).

https://doi.org/10.1016/j.neuroscience.2017.11.055
0306-4522/

ADVANCED NEUROIMAGING FOR THE NON-INVASIVE CHARACTERIZATION OF BRAIN STRUCTURE/FUNCTION

Magnetic resonance imaging (MRI) is a non-invasive technique capable of providing information regarding functional and structural brain properties that can be readily translated between preclinical and clinical settings. Recent advances in the acquisition and analysis of data widen the scope of this technique to increase our knowledge on neuroinflammatory diseases in general (see this Special Issue), and on the gut-brain axis in particular.

Functional MRI, with blood oxygen level-dependent contrast, provides readouts of neuronal activity across the brain (Moreno et al., 2013). When recorded normally and not in conjunction with task-associated paradigms, the so-called resting-state fMRI allows us to measure statistical interdependencies (normally correlations) between the signals recorded in selected brain regions, and to build global brain networks based on the interactions identified. Aided by analytical tools drawn from graph theory, a number of network characteristics (degree, clustering, centrality measures, etc.) can be also calculated to provide a more complete description of the brain's functional connectivity on a mesoscopic scale (Sporns and Zwi, 2004; Bullmore and Sporns, 2009). Significantly, the same graph theory tools are being used to characterize metabolic, genomic and proteomic networks of the gut microbiota (Layeghifard et al., 2017). Parameters of functional connectivity can serve to identify alterations in neurological and psychiatric diseases (Greicius, 2008; Menon, 2011), and altered fMRI data have been associated with alterations to the microbiota (Tillisch et al., 2008; Tillisch and Labus, 2014). When looking at elderly patients, for instance, a complex interaction between cognition, fMRI activation patterns and abundance of gut populations arises (Bajaj et al., 2016).

As to whether we can identify functional associations between brain and microbial networks in this way, the data currently available strongly suggest that combining these two 'omics' datasets in appropriate analysis pipelines will allow biological meaning to be extracted.

From a structural point of view, increasing specificity to different tissue sub-compartments (glia vs. neurons, soma vs. dendrites, axon diameter vs. myelin thickness or axonal density) opens the possibility of unmasking subtle microstructural changes in pathological states. For example, diffusion MRI, based on the random motion of water molecules in brain parenchyma (Basser et al., 1994) provides information on the orientation, size and volume fraction of axons in the white matter (Assaf et al., 2004; Barazany et al., 2009), and of dendrites in the gray matter (Jespersen et al., 2007). Combined with relaxometry (Mottershead et al., 2003) and magnetization transfer (Sled and Pike, 2000), diffusion can also quantify compartment-specific myelin contrast (De Santis et al., 2016). These methods have helped highlight early microstructural changes in multiple sclerosis that remained invisible with other techniques (De Santis et al., 2017). A pioneer work (Ahluwalia et al., 2014) has found repaired microstructure in fronto-parietal white matter, as measured with diffusion MRI, in cirrhosis patients after antibiotic treatment, along with a significant improvement in cognition including working memory and inhibitory control; a more recent paper (Ahluwalia et al., 2016) further reported significant correlation between diffusion MRI parameters and different gut microbial families in cirrhotic patients. Advanced and more specific/multimodal imaging parameters are expected to increase our ability to determine whether we can associate characteristic microstructural patterns in the brain with particular gut dysbiotic states, or *vice versa*.

Furthermore, different imaging modalities can be combined to characterize the brain parenchyma from distinct perspectives. It was recently shown that combining multi-parametric maps into a machine learning platform provides exceptional specificity, selectivity and sensitivity to identify subtle alterations to the brain parenchyma in an animal model of alcohol use disorders (Cosa et al., 2016), even those induced by just one month of alcohol consumption. Furthermore, a specific brain signature was identified that was associated to abstinence under naltrexone medication (Cosa et al., 2016). These fingerprints remained unnoticed by conventional unimodal imaging techniques when used in isolation, demonstrating the importance of employing multiple modalities together in order to obtain new perspectives of brain pathology. Hence, we must ascertain whether brain fingerprints of disease states can be associated to a set of metabolic, genomic or proteomic features of the patient's microbiota. Such data could serve to refine diagnoses and to establish accurate predictions on disease progression or treatment efficacy.

COMBINING BRAIN AND GUT DATA IN THE ERA OF BIG DATA

Methods have been developed for the high-throughput extraction of quantitative features from large amounts of imaging data, known as *radiomics* (Gillies et al., 2016), and these have been used to identify MRI biomarkers as objective characteristics extracted from medical images and related to normal or abnormal biological processes. Simultaneously, molecular techniques targeting the bacterial 16S rRNA gene have remarkably advanced the study of microbial composition; next-generation sequencing enable fast and high-throughput analyses at a reasonable cost, which is expected to expanding the knowledge of this complex ecosystem (Costello et al., 2009; Rintala et al., 2017). Comparable strategies as the ones used in radiomics to reduce dimensionality and for feature extraction are also being applied to the large amounts of information gathered from microbiome sequencing efforts (Gonzalez and Knight, 2012). From a systems (bio)-medicine perspective, associations between brain imaging and microbiota biomarkers have yet to be developed, although establishing such relationships could dramatically advance our understanding of the gut-brain axis (Tillisch and Labus, 2014; see scheme in Fig. 1 representing possible approaches to combine imaging and microbiota data). For instance, a vector of

Please cite this article in press as: De Santis S et al. Radiomicrobiomics: Advancing Along the Gut-brain Axis Through Big Data Analysis. Neuroscience (2017), https://doi.org/10.1016/j.neuroscience.2017.11.055

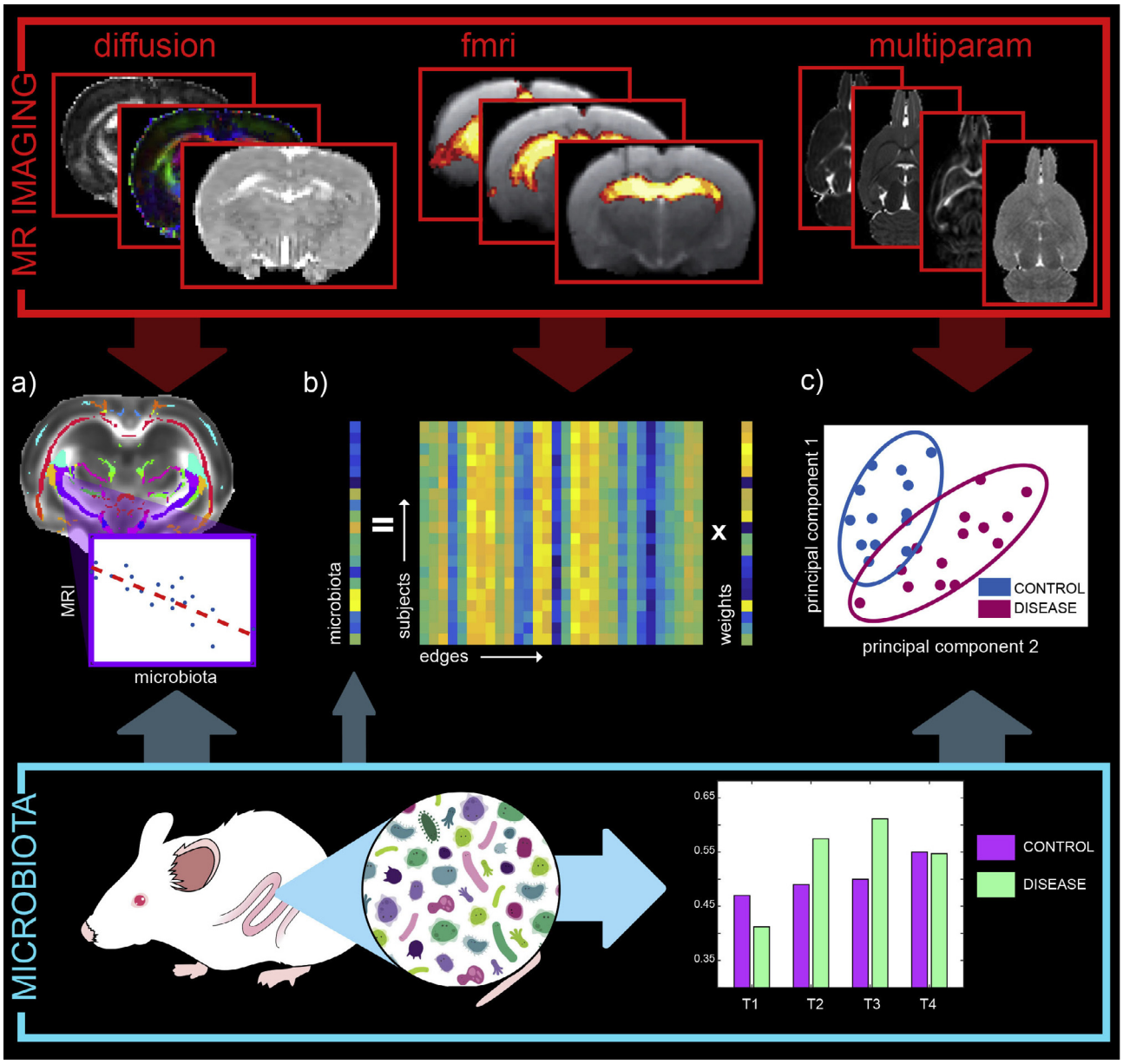

Fig. 1. Possible frameworks for combining imaging and microbiota data. (A) Examples of distinct MRI maps: color-coded principal direction weighted by fractional anisotropy from diffusion MRI (left); activation map from fMRI (centre); and multi-parametric acquisition with T1, T2, fractional anisotropy and mean diffusivity maps (right). (B) Example of correlation analysis between microstructure and microbiota in regions of interest, which can be defined using prior anatomical or functional information. (C) The connectivity matrix ($N_{subjects} \times N_{edges}$, each line representing the strength of the edge between two nodes) obtained by graph theory could be used to explain the microbiota composition of each subject through the regression coefficients calculated (adapted from Smith et al., 2013). (D) The hyperplane separating two conditions (experimental and control) in a machine learning analysis based on a supported vector machine framework. (E) Analysis of the microbiota in the rat intestine. The right plot represents the concentration of one microbiota population at different times (t1–t4) in two groups: control and experimental.

multimodal imaging features that identifies a specific pathological brain state has been applied to alcohol use disorders (i.e. 'post-alcohol exposure' or 'naltrexone-res ponding' states). Such an approach could also be used to mine microbiota datasets in order to first identify covariation, and then, to search for causality. Similarly, features of dysbiosis in the microbiota, either those that are as simple as the ratio between bacterial populations or some more complex features (e.g., network descriptors of the microbioma), could help identify the targets or causes in the brain for such a dysbiotic state, with single voxel imaging resolution (submillimetric). In the context of precision medicine, one can imagine taking decisions on treatment-selection informed by a comparable analysis of the microbiota in an individual, thereby maximizing the probability of a positive neurological outcome.

This practice is what we term here *radiomicrobiomics*: a process designed to extract quantitative parameters from the gut-brain axis by combining brain imaging and features of the microbiota, mining these data to

generate and/or to test hypotheses, and thereby developing decision support tools associated with disease biomarkers or treatment efficacy. Mathematical algorithms, computational methods and visualization tools have been already developed to combine 'omics' data of different biological origins (González et al., 2012; Smith et al., 2013). These will help to generalize trends in the data from partial samples and construct biomarkers that permit dysfunctional states in the gut-brain axis to be defined. Combining features from different MRI modalities already opens the window to precisely identify brain alterations (Cosa et al., 2016). Furthermore, by identifying the most informative features, those that best characterize the conditions studied, their causes and effects on the gut-brain axis could be disentangled using animal models.

CONCLUDING REMARKS

The automatic identification of models and patterns of interactions in the gut-brain axis by combining image-based brain biomarkers with features of the microbiota, or *radiomicrobiomics*, has tremendous potential to enhance our understanding of this fascinating connection. Comprehensive datasets mined with big data tools and current experimental capabilities to assess causality (i.e. transplants of microbiota and optogenetics to manipulate the brain), may facilitate studies that will reveal the rules governing bidirectional communication along the conduit between these two organs.

Acknowledgments—These studies were supported in part by the Spanish Ministerio de Economía y Competitividad (MINECO) and FEDER funds under grants BFU2015-64380-C2-1-R (S.C.) and BFU2015-64380-C2-2-R (D.M.). This project received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 668863. S.C. acknowledges financial support from the Spanish State Research Agency, through the "Severo Ochoa" Programme for Centres of Excellence in R&D (ref. SEV- 2013-0317). D.M. acknowledges financial support from the Conselleria d'Educació, Investigació, Cultura i Esport, Generalitat Valenciana (grant AEST/2017/013). S.D.S was supported by a NARSAD Young Investigator Grant (Grant #: 25104).

REFERENCES

Ahluwalia V, Wade JB, Heuman DM, Hammeke TA, Sanyal AJ, Sterling RK, Stravitz RT, Luketic V, Siddiqui MS, Puri P, Fuchs M, Lennon MJ, Kraft KA, Gilles H, White MB, Noble NA, Bajaj JS (2014) Enhancement of functional connectivity, working memory and inhibitory control on multi-modal brain MR imaging with Rifaximin in Cirrhosis: implications for the gut-liver-brain axs. Metab Brain Dis 29(4):1017–1025.

Ahluwalia V, Betrapally NS, Hylemon PB, White MB, Gillevet PM, Unser AB, Fagan A, Daita K, Heuman DM, Zhou H, Sikaroodi M, Bajaj JS (2016) Impaired gut-liver-brain axis in patients with cirrhosis. Sci Rep 6:26800.

Assaf Y, Freidlin RZ, Rohde GK, Basser PJ (2004) New modeling and experimental framework to characterize hindered and restricted water diffusion in brain white matter. Magn Res Med 52:965–978.

Bajaj JS, Ahluwalia V, Steinberg JL, Hobgood S, Boling PA, Godschalk M, Habib S, White MB, Fagan A, Gavis EA, Ganapathy D, Hylemon PB, Stewart KE, Keradman R, Liu EJ, Wang J, Gillevet PM, Sikaroodi M, Moeller FG, Wade JB (2016) Elderly patients have an altered gut-brain axis regardless of the presence of cirrhosis. Sci Rep 6:38481.

Barazany D, Basser PJ, Assaf Y (2009) In vivo measurement of axon diameter distribution in the corpus callosum of rat brain. Brain 132:1210–1220.

Basser PJ, Mattiello J, Le Bihan D (1994) Estimation of the effective self-diffusion tensor from the NMR spin echo. J Magn Reson 103:247–254.

Bullmore E, Sporns O (2009) Complex brain networks: graph theoretical analysis of structural and functional systems. Nat Rev Neurosci 10:186–198.

Cosa A, Moreno A, Pacheco-Torres J, Ciccocioppo R, Hyytiä P, Sommer WH, Moratal D, Canals S (2016) Multi-modal MRI classifiers identify excessive alcohol consumption and treatment effects in the brain. Addict Biol. https://doi.org/10.1111/adb.12418.

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R (2009) Bacterial community variation in human body habitats across space and time. Science 326(5960):1694–1697.

De Santis S, Jeurissen B, Jones DK, Assaf Y, Roebroeck A (2016) T1 relaxometry of crossing fibres in the human brain. Neuroimage 141:133–142.

De Santis S, Granberg T, Ouellette R, Treaba CA, Fan Q, Herranz E, Mainero C, Toschi N (2017) Early axonal damage in normal appearing white matter in Multiple Sclerosis: novel insights from multi-shell diffusion MRI. In: 39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Jeju Island, Korea, July 11–15.

Dinan TG, Cryan JF (2012) Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. Psychoneuroendocrinology 37:1369–1378.

Dinan TG, Cryan JF (2017a) Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. J Physiol 595:489–503.

Dinan TG, Cryan JF (2017b) Gut-brain axis in 2016: brain-gut-microbiota axis - mood, metabolism and behaviour. Nat Rev Gastroenterol Hepatol 14:69–70.

El Aidy S, Dinan TG, Cryan JF (2014) Immune modulation of the brain-gut-microbiota axis. Front Microbiol 5:146.

Gillies RJ, Kinahan PE, Hricak H (2016) Radiomics: images are more than pictures, they are data. Radiology 278:563–577.

Gonzalez A, Knight R (2012) Advancing analytical algorithms and pipelines for billions of microbial sequences. Curr Opin Biotechnol 23:64–71.

González I, Cao KL, Davis MJ, Déjean S (2012) Visualising associations between paired 'omics' data sets. BioData Mining 5:19.

Greicius M (2008) Resting-state functional connectivity in neuropsychiatric disorders. Curr Opin Neurol 21:424–430.

Jespersen SN, Kroenke CD, Østergaard L, Ackerman JJ, Yablonskiy DA (2007) Modeling dendrite density from magnetic resonance diffusion measurements. Neuroimage 34:1473–1486.

Kamada N, Chen GY, Inohara N, Nuñez G (2013) Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 14:685–690.

Kelly JR, Borre Y, O'Brien C, Patterson E, El Aidy S, Deane J, Kennedy PJ, Beers S, Scott K, Moloney G, Hoban AE, Scott L, Fitzgerald P, Ross P, Stanton C, Clarke G, Cryan JF, Dinan TG (2016) Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. J Psychiatr Res 82:109–118.

Layeghifard M, Hwang DM, Guttman DS (2017) Disentangling interactions in the microbiome: a network perspective. Trends Microbiol 25:217–228.

Menon V (2011) Large-scale brain networks and psychopathology: a unifying triple network model. Trends Cogn Sci 15:483–506.

Moreno A, Jego P, de la Cruz F, Canals S (2013) Neurophysiological, metabolic and cellular compartments that drive neurovascular coupling and neuroimaging signals. Front Neuroenergetics 28(5):3.

Mottershead JP, Schmierer K, Clemence M, Thornton JS, Scaravilli F, Barker GJ, Tofts PS, Newcombe J, Cuzner ML, Ordidge RJ, McDonald WI, Miller DH (2003) High field MRI correlates of myelin content and axonal density in multiple sclerosis-a post-mortem study of the spinal cord. J Neurol 250:1293–1301.

Ochoa-Repáraz J, Kasper LH (2017) The influence of gut-derived CD39 regulatory T cells in CNS demyelinating disease. Transl Res 179:126–138.

Rintala A, Pietilä S, Munukka E, Eerola E, Pursiheimo JP, Laiho A, Pekkala S, Huovinen P (2017) Gut microbiota analysis results are highly dependent on the 16S rRNA gene target region, whereas the impact of DNA extraction is minor. Biomol Tech 28(1):19–30.

Sled JG, Pike GB (2000) Quantitative interpretation of magnetization transfer in spoiled gradient echo MRI sequences. J Magn Reson 145:24–36.

Smith SM, Vidaurre D, Beckmann CF, Glasser MF, Jenkinson M, Miller KL, Nichols TE, Robinson E, Salimi-Khorshidi G, Woolrich MW, Barch DM, Uğurbil K, Van Essen DC (2013) Functional connectomics from resting-state fMRI. Trends Cogn Sci 17:666–682.

Sporns O, Zwi JD (2004) The small world of the cerebral cortex. Neuroinformatics 2:145–162.

Tillisch K, Labus JS (2014) Neuroimaging the microbiome-gut-brain axis. Adv Exp Med Biol 817:405–416.

Tillisch K, Wang Z, Kilpatrick L, Holschneider DP, Mayer AC (2008) Studying the brain-gut axis with pharmacological imaging. Ann N Y Acad Sci 1144:256–264.

Zhao L (2013) The gut microbiota and obesity: from correlation to causality. Nat Rev Microbiol 11:639–647.

(Received 1 August 2017, Accepted 30 November 2017)
(Available online xxxx)