Microbiota-Gut-Brain Research: A Critical Analysis

Supplementary material

Long abstract

Microbiota-gut-brain (MGB) research is a fast-growing field of inquiry with important implications for how human brain function and behaviour are understood. Researchers manipulate gut microbes ('microbiota') to reveal connections between intestinal microbiota and normal brain functions (e.g., cognition, emotion, memory) or pathological states (e.g., anxiety and mood disorders, neural developmental disorders such as autism). Many claims are made about causal relationships between gut microbiota and human behaviour. By uncovering these relationships, MGB research aims to offer new explanations of mental health and potential avenues of treatment.

So far, limited evaluation has been made of MGB's methods and its core experimental findings, many of which are extensively reiterated in copious reviews of the field. These factors, plus the self-help potential of MGB, have combined to encourage uncritical public uptake of MGB discoveries. Both social and professional media focus on the potential for dietary intervention in mental health, and causal relationships are assumed to be established.

Our target article has two main aims. One is to examine critically the core practices and findings of experimental MGB research, and to raise questions about them for brain and behavioural scientists who may not be familiar with the field. The other is to challenge the way in which MGB findings are presented. Our positive goal is to suggest how current problems and weaknesses may be addressed, in order for both scientific and public audiences to gain a clearer picture of MGB research and its strengths and limitations.

1. MGB literature

Proportion of review articles

There is a very high proportion of review articles in the MGB field, whichever search term is used.

- "Gut brain bacteria" finds 326 reviews out of 768 articles (43%)
- "Gut-brain microbiota" finds 167 reviews out of 325 articles (51%)
- "Microbiota brain" finds 386 reviews out of 865 articles (45%).

Those numbers only include whatever PubMed automatically lists as a review. The true numbers are probably higher. We discarded the reviews that turned up in our highly cited list in order to get at the core experimental corpus of the field.

Most prolific authors

The five most prolific authors in the field as of 22.05.2017 (represented by the 867 source articles):

1. John F Cryan (81 articles, including >43 reviews)
2. Timothy/Ted G Dinan (79 articles, including >44 reviews)
3. Gerard Clarke (29 articles, including >17 reviews)
4. John Bienenstock (21 articles, including >6 reviews)
5. Premysl Bercik (19 articles, including >9 reviews)

Cryan (a neuropharmacologist) and Dinan (a pharmacologist/psychiatrist) are both leaders of gut-brain microbiota axis at the APC Microbiome Institute (University College Cork, Ireland) and have authored 222 papers together. They have also published with Clarke, a neuropharmacologist/psychiatrist at UCC. Bienenstock, an immunologist (McMaster, Canada) and Bercik (McMaster), a neural gastroenterologist, are not directly connected to this group.

Supplementary Table 1: The broad range of MGB-associated methods, with exemplar publications selected from 25 most-cited MGB experimental papers (see Section 4).

General method	Specific features of methods
Animal models	• Germ-free (GF), specific pathogen free (SPF), and conventionalized rodents (e.g., Sudo et al. 2004) • Autism model rats (e.g., de Theije et al. 2014) • Colitis model rodents (e.g., Ohland et al. 2013) • Depression model rodents (e.g., Park et al. 2013) • Maternal immune activation rodents (e.g., Hsiao et al. 2013) • Pups undergoing maternal separation (e.g., O'Mahony et al. 2009)
Animal behavioural tests	• Forced swim (e.g., Bravo et al. 2011) • Step down (e.g., Bercik et al. 2011) • Elevated plus maze (e.g., Neufeld et al. 2011b) • Light-dark choice (e.g., Gareau et al. 2011) • Open field (e.g., Diaz Heijtz et al. 2011) • Object recognition (e.g., Desbonnet et al. 2015) • Social interaction (e.g., Hsiao et al. 2013)
Rodent gut, brain and blood biochemical assays	• Neurochemical brain analysis and blood metabolites by high-performance liquid chromatography (e.g., Clarke et al. 2013) • Microbial metabolites in faeces by gas chromatography (e.g., Leclercq et al. 2014) • Measurements of neurotrophin, nerve growth factor protein (e.g., Sudo et al. 2004), C-reactive protein (e.g., Leclercq et al. 2014), hormones (e.g., Bruce-Keller et al. 2015), cytokines and chemokines by immunoassays (e.g., Ohland et al. 2013) • Intestinal permeability assays (e.g., Hsiao et al. 2013) • Blood endotoxin tests (e.g., Ait-Belgnaoui et al. 2014)
Human behavioural assessments	• Human patient self-reports, depression and anxiety scales (e.g., Messaoudi et al. 2011; Steenbergen et al. 2015)
Experimental interventions in rodents	• Probiotics (e.g., Tillisch et al. 2013) • Microbiota transplants (e.g., Diaz Heijtz et al. 2011) • Antibiotic treatments (e.g., Bercik et al. 2011) • Dietary alterations (e.g., Ohland et al. 2013) • Pathogen introduction (e.g., Gareau et al. 2011) • Vagotomy (e.g., (Bravo et al. 2011)

Experimental interventions in humans	• Probiotics and dietary interventions (e.g., Tillisch et al. 2013) • Antibiotic treatments (e.g., Bajaj et al. 2013)
Microbiome analyses	• Comparisons of gut microbiomes of mice/humans with brain or behavioural disorders to the microbiome of healthy organisms (e.g., Bailey et al. 2011; Jiang et al. 2015) • Comparison of host microbiomes before and after interventions (e.g., Messaoudi et al. 2011; Tillisch et al. 2013)

Supplementary Table 2: Probiotic methods in 25 most cited MGB publications

Publication	**Probiotic**	**Model**
Bravo et al. (2011)	*Lactobacillus rhamnosus*	BALB/c mice
Messaoudi et al. (2011)	*Lactobacillus helveticus* R0052 *Bifidobacterium longum* R0175	Wistar rats Human (clinical trial)
Gareau et al. (2011)	*Lactobacillus rhamnosus* R0011 *Lactobacillus helveticus* R0052	C57BL/6 mice
Ait-Belgnaoui et al. (2012)	*Lactobacillus farciminis*	Wistar rats
Hsiao et al. (2013)	*Bacteroides fragilis*	C57BL/6N mice
Ohland et al. (2013)	*Lactobacillus helveticus*	129/SvEv mice
Ait-Belgnaoui et al. (2014)	*Lactobacillus helveticus* R0052 *Bifidobacterium longum* R0175	C57Bl6 mice
Steenbergen et al. (2015)	*Bifidobacterium bifidum* W23 *Bifidobacterium lactis* W52 *Lactobacillus acidophilus* W37 *Lactobacillus brevis* W63 *Lactobacillus casei* W56 *Lactobacillus salivarius* W24 *Lactococcus lactis*	Human (clinical trial)

Supplementary Table 3: Microbiome methods in the 25 most cited MGB publications

Publication	Method	Comment
O'Mahony et al. (2009)	DGGE	Detects different profiles between two groups; an older qualitative method.
Bercik et al. (2011)	Culture DGGE + Sanger sequencing	Detects different profiles between two groups; some specific taxa listed; an older qualitative method. Sanger sequencing superseded.
Bailey et al. (2011)	V4-V6 16S rRNA 454-pyrosequencing	Superseded sequencing and analysis methods, with a small number of reads obtained per sample. Some evidence for differences at time 0 from clustering, but not narrowed down to specific taxa.
Gareau et al. (2011)	16S rRNA qPCR	Some taxa are estimated by qPCR. No mention of the exact normalization performed (e.g., Is rRNA copy number taken into account?).
Bajaj et al. (2013)	V1-V2 16S rRNA GS-Junior pyrosequencing	No significant changes in profile are reported. A correlation network was built for microbiome and metabolome data, and connectivity of the network was compared.
Park et al. (2013)	DGGE	Detection of different profiles between two groups via an older qualitative method.
Hsiao et al. (2013)	V3-V5 16S rRNA 454-pyrosequencing	Appropriate bioinformatic and statistical tools used.
Tillisch et al. (2013)	V5-V6 16S rRNA 454-pyrosequencing	"Post-hoc analysis of fecal microbiota composition indicated a good randomization of the subjects at baseline. No significant change in microbiota composition vs baseline was found after intervention between groups" (p. 1397). In other words, the study did not observe any change in the microbiota.

Ohland et al. (2013)	T-RFLP	An old-fashioned method that flattens the diversity in the sample to small number of distinguishable peaks. That flattening might be the reason for a very clear separation of the samples on the PCA plot.
Leclercq et al. (2014)	V1-V2 16S rRNA 454-pyrosequencing 16S rRNA qPCR	Appropriate bioinformatic and statistical tools used.
de Theije et al. (2014)	V3-V5 16S rRNA 454-pyrosequencing	Appropriate bioinformatic and statistical tools used.
Jiang et al. (2015)	V1-V3 16S rRNA 454-pyrosequencing	Appropriate bioinformatic and statistical tools used but no beta-diversity/PCA presentation in the main figures, due to high variation. Instead, the authors perform multiple tests with the aim of finding significant taxa.
Bruce-Keller et al. (2015)	V3-V4 16S rRNA Illumina MiSeq sequencing	Appropriate bioinformatic and statistical tools used.

Abbreviations
DGGE: denaturing gel electrophoresis; T-RFLP: terminal restriction fragment length polymorphism; V1-V6 16S rRNA: 16S ribosomal RNA variable regions 1-6.

Supplementary Tables 4a-d: Five categories of central nervous system-related foci and methods in 25 most cited MGB papers

Supplementary Table 4a: Neuroendocrine "stress" axis

Publication	HPA-axis assessment	Species
Bravo et al. (2011)	Forced swim, Cort	BALB/c mice
Sudo et al. (2004)	Restraint, CRH, ACTH, Cort	BALB/c mice
O'Mahony et al. (2009)	Maternal separation, Cort	Sprague Dawley rats
Neufeld et al. (2011b)	Cort 48 h after arrival in lab	Swiss Webster mice
Messaoudi et al. (2011)	Urinary 24 h Cort	Human
Clarke et al. (2013)	Novel cage, Cort	Swiss Webster mice
Gareau et al. (2011)	Water avoidance stress, Cort	C57BL/6 mice
Ait-Belgnaoui et al. (2012)	Restraint, CRH, ACTH, Cort	Wistar rats
Crumeyrolle-Arias et al. (2014)	Open field, CRH, Cort	F344 rats
Ohland et al. (2013)	Brain and fecal Cort	129/sVEv mice
Park et al. (2013)	Water avoidance stress, CRH	C57BL/6 mice
Ait-Belgnaoui et al. (2014)	Water avoidance stress, Cort, c-Fos PVH	C57Bl6 mice
Desbonnet et al. (2015)	Restraint, Cort	NIH Swiss mice

Abbreviations
ACTH: adrenocorticotropic hormone; c-Fos: immediate early gene product often used as cellular activation marker; Cort: corticosterone; CRH: corticotropin-releasing hormone; PVH: paraventricular nucleus of the hypothalamus

Supplementary Table 4b: Emotion-mood: Anxiety

Publication	Anxiety assessment	Species
Diaz Heijtz et al. (2011)	Light-dark box, elevated plus maze, open field	NMRI mice
Hsiao et al. (2013)	Open field	C57BL/6N mice
Bravo et al. (2011)	Elevated plus maze, fear conditioning, open field	BALB/c mice
Bercik et al. (2011)	Light-dark box, step down test	BALB/c mice
O'Mahony et al. (2009)	Open field fecal boli	Sprague Dawley rats
Neufeld et al. (2011b, 2011a)	Elevated plus maze	Swiss Webster mice
Messaoudi et al. (2011)	Conditioned defensive probe burying HADS anxiety subscale	Wistar rats Human
Clarke et al. (2013)	Light-dark box	Swiss Webster mice
Gareau et al. (2011)	Water avoidance stress, light-dark box	C57BL/6 mice
Leclercq et al. (2014)	State-trait anxiety inventory	Alcohol-dependent humans
Steenbergen et al. (2015)	Beck anxiety inventory	Human
Bruce-Keller et al. (2015)	Elevated plus maze, fear conditioning, open field	C57BL/6 mice
Crumeyrolle-Arias et al. (2014)	Open field	F344 rats
Ohland et al. (2013)	Barnes maze	129/sVEv mice
Park et al. (2013)	Open field, step down test	C57BL/6 mice
Desbonnet et al. (2015)	Light-dark box	NIH Swiss mice

Abbreviations
HADS: Hospital Anxiety and Depression Scale

Supplementary Table 4c: Mood disorder: Depression

Publication	Depression assessment	Species
Bravo et al. (2011)	Forced swim test	BALB/c mice
Messaoudi et al. (2011)	HADS depression subscale	Human
Jiang et al. (2015)	DSM, Hamilton's depression scale, Montgomery–Asberg Depression Rating Scale	Human
Leclercq et al. (2014)	Beck depression inventory	Alcohol-dependent humans
Steenbergen et al. (2015)	LEIDS-r, Beck depression inventory	Human
Park et al. (2013)	Tail suspension test	C57BL/6 mice

Abbreviations
DSM: Diagnostic and Statistical Manual of Mental Disorders

Supplementary Table 4d: Autism spectrum/developmental disorders

Publication	Autism spectrum assessment	Species
Hsiao et al. (2013)	Pre-pulse inhibition, marble burying, social interaction	C57BL/6N mice
de Theije et al. (2014)	Social interaction	BALB/c mice

Supplementary Table 4e: Cognition

Publication	Cognition assessment	Species
Bravo et al. (2011)	Fear conditioning	BALB/c mice
Gareau et al. (2011)	Novel object recognition, T-maze	C57BL/6 mice
Bajaj et al. (2013)	BDT and the psychometric hepatic encephalopathy score (PHES; consists of NCT-A, NCT-B, DST, line tracing test and serial dotting)	Cirrhotic humans
Bruce-Keller et al. (2015)	Fear conditioning	C57BL/6 mice
Ohland et al. (2013)	Elevated Barnes maze	129/sVEv mice
Desbonnet et al. (2015)	Novel object recognition, social transmission food preference (social memory)	NIH Swiss mice

5. Terminology evaluation

When addressing anxiety and depression in animals, it is important to keep in mind that these categories depend on verbal reports of feelings in humans and can therefore not be assessed in the same way in animals. It can even be questioned if animals experience anxiety and depression, even though we can readily observe behavioural and physiological changes in test situations that we consider would induce fear or giving up an active coping strategy. To emphasize the distinction between human anxiety and depression and what scientists can assess in animals, it is considered good scientific practice to describe the results of such behavioural tests with terms such as “anxiety-like” or “depression-like” rather than directly inferring anxiety and depression. As shown in tables 5a and 5b, all MGB animal studies relevant to anxiety and depression employ appropriate “anxiety-like” and “depression-like” descriptive terms, even though they all also discuss anxiety and depression as such.

Supplementary Table 5a: Behavioural tests and descriptive terms relative to anxiety

Publication	Behavioural test	Descriptions
Diaz Heijtz et al. (2011)	Light-dark box, elevated plus maze, open field	Anxiety, anxiety behaviour, anxiety-like behaviour
Hsiao et al. (2013)	Open field	Anxiety, anxiety-associated, anxiety-like behaviour
Bravo et al. (2011)	Elevated plus maze, fear conditioning, open field	Anxiety, anxiety behaviour, anxiety-like behaviour, anxiety-related, anxiety responses
Bercik et al. (2011)	Light-dark box, step down test	Anxiety, anxiety-like behaviour
O’Mahony et al. (2009)	Open field fecal boli	Anxiety, anxiety-like behaviour
Neufeld et al. (2011a, 2011b)	Elevated plus maze	Anxiety, anxiety-like behaviour
Messaoudi et al. (2011)	Conditioned defensive probe burying	Anxiety, anxiety-like behaviour, anxiety related
Clarke et al. (2013)	Light-dark box	Anxiety, anxiety behaviour, anxiety-like behaviour
Gareau et al. (2011)	Water avoidance stress, light-dark box	Anxiety, anxiety-like behaviour
Bruce-Keller et al. (2015)	Elevated plus maze, fear conditioning, open field	Anxiety, anxiety behaviour, anxiety-based behaviour, anxiety-like behaviour
Crumeyrolle-Arias et al. (2014)	Open field	Anxiety, anxiety-like behaviour, anxiety-like response, signs of anxiety
Ohland et al. (2013)	Elevated Barnes maze	Anxiety, anxiety behaviour, anxiety-like behaviour
Park et al. (2013)	Open field, step down test	Anxiety, anxiety-like behaviour
Desbonnet et al. (2015)	Light-dark box	Anxiety, anxiety behaviour

Supplementary Table 5b: Behavioural tests and descriptive terms relative to depression

Publication	Behavioural test	Descriptions
Bravo et al. (2011)	Forced swim test	Depression, depression-related behaviour
Park et al. (2013)	Tail suspension test	Depression, depression-like behaviour; behavioural depression

In addition to scoring the occurrences of descriptive terms, we also assessed the context in which they were used. We found several instances of inappropriate descriptive terms. With respect to the open field test, for example, Hsiao et al. (2013) write that: "Open field exploration involves mapping an animal's movement in an open arena to measure locomotion and anxiety (Bourin et al., 2007)", and Bravo et al. (2011) report that "*L. rhamnosus* (JB-1)-fed mice were less anxious". Similarly for depression, Park et al. (2013) state that "The induction of chronic depression alters motor activity and the microbial profile in the colon", and Jiang et al. (2015) note that "Studies using animal models have shown that depression affects the stability of the microbiota". Thus, even though all studies in our sample do use "anxiety-like" and "depression-like" descriptive terms when describing findings obtained in animal behavioural tests, several of them also employ anxiety and depression in an inappropriate way, namely to describe anthropomorphically phenomena derived from rodent models.

Supplementary Table 6: Independent variables tested, plus statistical methods used in the 25 most cited MGB papers. **Bold font** in the statistical-test cells indicates seemingly inappropriate use of statistical tests in view of the experimental design.

Publication	Independent variables	Statistical test
Diaz Heijtz et al. (2011)	Microbiota status (GF vs SPF)	Repeated measures ANOVA (phenotype between, time within) One-way ANOVA
Hsiao et al. (2013)	Maternal immune activation; *B. fragilis* treatment	**Student's t-test, one way ANOVA, two-way repeated measure ANOVA (PPI, CD4+ stim)**
Bravo et al. (2011)	Ingestion of *Lactobacillus;* Vagotomy	Student's t-test, two-way ANOVA
Sudo et al. (2004)	Microbiota status (GF, SPF and gnotobiotic); Stress (restraint, ether); Reconstitution with bacteria	**Factorial analysis of variance**
Bercik et al. (2011)	Microbiota status (antibiotic); Recolonization; Nerve transection	**Analysis of variance**

O'Mahony et al. (2009)	Early life stress (Open field, but all animals) (Colorectal distension, but all animals)	Student's t-test, two-way ANOVA (CRD, open field)
Neufeld et al. (2011a, 2011b)	Microbiota status (GF vs SPF; colonized GF vs SPF)	Student's t-test, **two-way ANOVA (OF, EPM)**
Tillisch et al. (2013)	Consumption of probiotics	T test
Messaoudi et al. (2011)	Consumption of probiotics; Diazepam (Conditioned defensive burying, but all animals)	Kruskal-Wallis rank sum test, Mann-Whitney U test, Wilcoxon test
Bailey et al. (2011)	Social disruption stress; Antibiotic	Two-way ANOVA
Clarke et al. (2013)	Microbiota status (GF, conventional and colonized GF); Novelty stress Sex	One-, two- and three-way ANOVA
Gareau et al. (2011)	Infection (*Citrobacter rodentium*); Water avoidance stress; Probiotic treatment; Microbiota status (GF vs conventional)	Student t-test, *one*-, two-way ANOVA, Mann-Whitney test
Ait-Belgnaoui et al. (2012)	Partial restraint stress; *L. farciminis* treatment; Myosin light chain kinase inhibitor; Antibiotic treatment	**Student's t-test**
Bajaj et al. (2013)	Rifaximin treatment; Time (before and after treatment)	Student t-test, principal coordinate analysis (PCA), Wilcoxon matched-pair signed rank tests
Jiang et al. (2015)	Depression (active MDD, respond MDD, healthy control	Kruskal-Wallis rank sum test
Leclercq et al. (2014)	Alcohol dependent detoxification before and after; Intestinal permeability (high, low)	**Parametric ANOVA**, Kruskal-Wallis, Wilcoxon or paired Student t-tests
de Theije et al. (2014)	Valproic acid injection during gestation; Sex	Kruskal-Wallis rank sum test, Mann-Whitney U test
Steenbergen et al. (2015)	Probiotic consumption (multispecies preparation vs placebo); Time (before)	Repeated measures ANOVA (treatment between, time within), Bayesian probabilities

Bruce-Keller et al. (2015)	Microbiota transfer (high fat or control diet-fed donors)	Student t-test, repeated measures two-way ANOVA (body weight, fear conditioning)
Crumeyrolle-Arias et al. (2014)	Microbiota status (GF vs SPF); Novelty stress (open field or not)	Student's t-test, two-way repeated measures ANOVA (microbial status between, time on sniffing within), Mann-Whitney and Kruskal
Ohland et al. (2013)	Genotype (WT vs IL-10 KO); Diet (standard chow vs Western); *Lactobacillus helveticus* gavage	**Student's one-way ANOVA**
Park et al. (2013)	Olfactory bulbectomy; Intracerebroventricular injection (CRH vs vehicle) (Water avoidance stress, but all animals)	Student's t-test
Ait-Belgnaoui et al. (2014)	Probiotic administration (*Lactobacillus helveticus* R0052 & *Bifidobacterium longum* R0175, *L. salivrius*, placebo); Chronic water avoidance stress	**One-way ANOVA**
Desbonnet et al. (2015)	Microbiota status (antibiotic) Restraint stress	Student t-test, factorial and repeated measures ANOVA (antibiotic and stress between, time within)

7. Microbiota patents and probiotics

We used the European Patent Office's search tool to find all patents written in English containing the words "microbiome OR microbiota" in the title, abstract or the full text. This search found 2096 patents, which we downloaded and analysed. There is inflationary growth in the number of patents awarded since mid-2016 (Fig. S1).

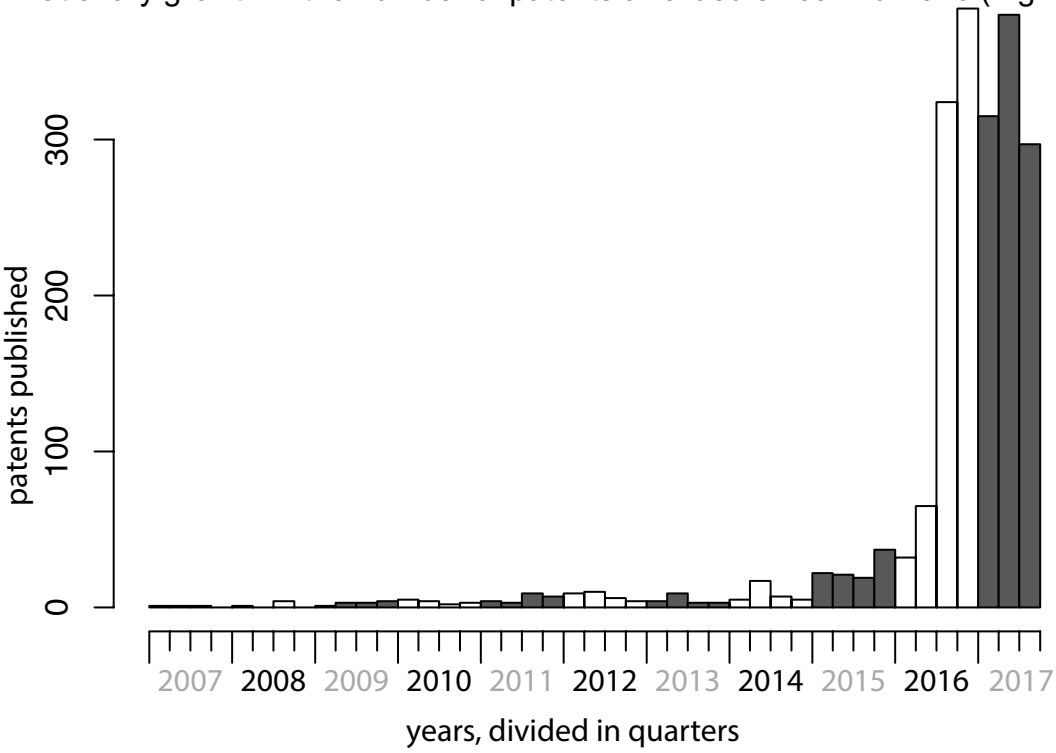


Figure S1. Histogram of the number of microbiota-related patents published in the last ten years.

The majority of microbiota patents in the EPO database are filed from the US,[1] and the contribution of the next most prolific country, France, is one-tenth that of US (Fig. S2A). Patent applicants vary from food companies and for-profit biotech companies to academic research institutions (Fig. S2). Elwha LCC, the second most prolific patent applicant, is a holding company with a large but latent patent portfolio. Its business model involves acquiring patents and never attempting to commercialize them, but instead using them to file lawsuits against institutions using those inventions.

[1] These patents would also be filed at the US Patent Office, which is larger than the EPO.

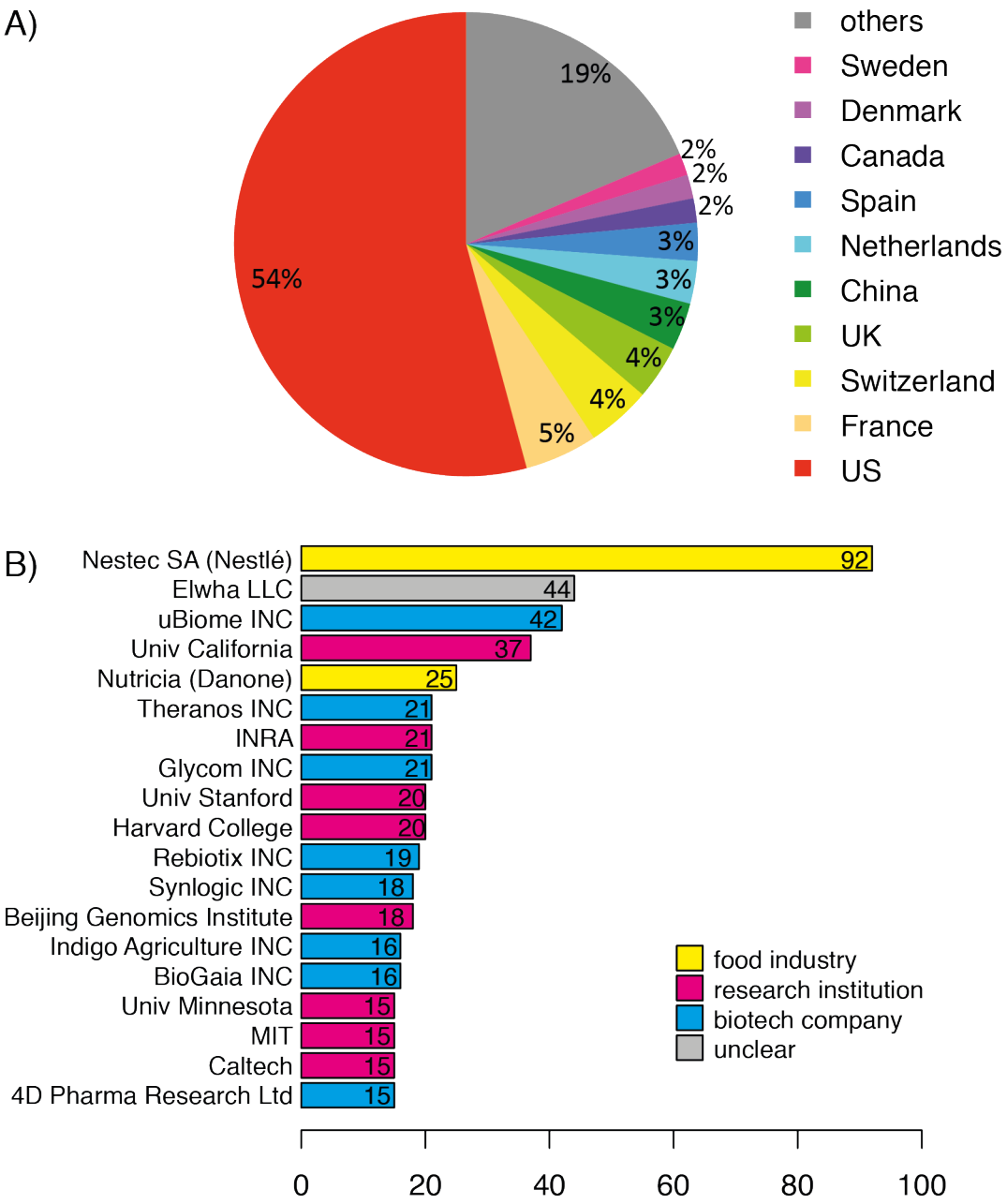


Figure S2. Microbiota patents applicants A) Country of origin of the microbiota related patents' applicants. B) The most prolific patent applicants.

As might be expected, the most popular class of microbiota patents is the category labelled C12Q1/68: "Measuring or testing processes involving enzymes, nucleic acids or microorganisms". The next largest proportion of patents involves administration or preparation of probiotics (class A61K3 and its subclasses).

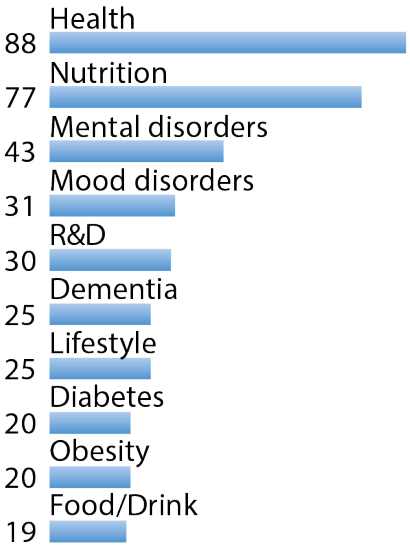
8. Microbiota gut brain in general press and online sources

We used Factiva (http://www.factiva.com) to search for mass media articles about MGB research. The search was performed on July 4th, 2018 with the term: "(microbiome OR microbiota) AND ((brain AND (cogn* OR (behavio* AND (mood OR stress)))) OR gut brain axis)". The search scoured English-language major news and business sources, and resulted in 310 articles. Among those articles, the most mentioned companies include University College Cork and Synthetic Biologics. The most prolific source was the Daily Mail with 46 articles, followed by The Times (UK) with 25 articles. US sources include Dow Jones Newswires and The New York Times (Fig. S3).

Figure S3. Most mentioned Subjects, Industries, Sources, Companies and Regions in the 310 general press articles pertaining to "microbiota gut brain" found through a Factiva search.

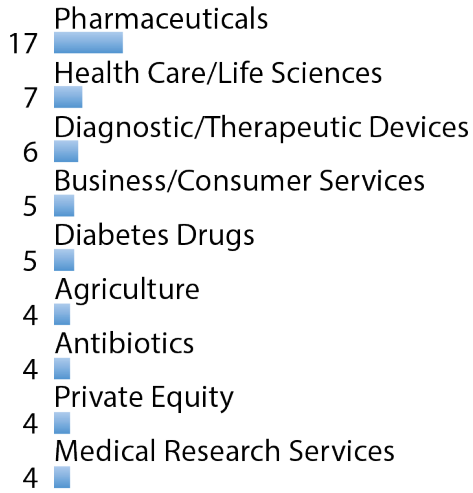
Most mentioned

Subjects

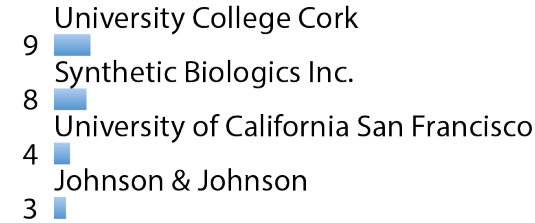


Sources

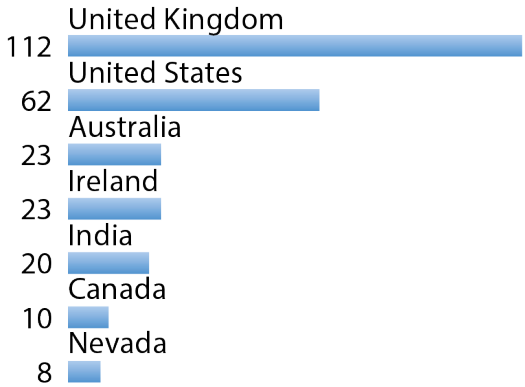
Industries



Companies



Regions



Supplementary Material Table 8: Selected quotes from online sites (in order of appearance in a list generated from a Google search for 'gut brain microbiome'). 50 hits were examined, scholarly articles discarded, and quotes extracted from the first 10 remaining sites.

'The microbiome-gut-brain axis also has the potential to fortify mental toughness in both sport and life'	Psychology Today Blog
'Recent findings between the gut microbiota and the brain suggest that our microbiota can deeply influence our health, brain and potentially even our behaviour. The microbiota has been associated with several neurological conditions and it could be playing a role in your migraine condition'	MigrainePal
'It is of course not surprising that gut microbiota profiling is not included as part of the routine clinical practice … Most psychiatric patients have digestive disorders, where 99% of all cognitive disorders start, so why are they being treated with Psychotropic drugs [and not diet?]'	Dr John Bergman
'Down in your large intestine live trillions and trillions of helpful bacteria and other microscopic creatures, collectively known as your gut microbiome. These critters help digest your food, keep you in good health, and — according to new research — influence your thoughts'	Nine Digital Coach
'It seems that our minds are, in some part, controlled by the bacteria in our bowels'	Medical News Today
'Gut flora influence various brain functions, affecting your thoughts, emotions, and memory'	Dr Lam Coaching
'What we eat or take can also weaken our microbiome. Pharmaceuticals such as anti-biotics, the oral contraceptive pill, antidepressants all wipe out our beneficial bacteria. Foods such as coffee, alcohol, refined sugar, Artificial and GMO Foods also disrupt the balance'	Griffith Consulting
'Research suggests that up to 90% of health conditions be [sic] linked to the microbiome and the gut. Find out which top foods to avoid in your diet and which to increase in order to create a healthy gut-brain connection. Learn all of the other ways, including supplementation, which can protect the microbiome. Your gut-brain axis also affects your memory, concentration and focus'	Institute of Holistic Nutrition
'This study suggests that reduced microbiome diversity may be related to weight gain in women taking atypical antipsychotics. Atypicals are in widespread use in the US. Given the other benefits of probiotic supplementation, it would be a winning proposition to add probiotics for those taking atypical antipsychotics'	Janet Settle Integrative Psychiatry

'The lack of diversity in the microbial population, and the integrity of the intestinal wall – which is maintained with a healthy, flourishing microbiome – may influence negative symptoms and disease such as depression, anxiety, autism and Parkinson's disease. Gut bacteria may also impact teen behavior, and appears to influence levels of mood modulating neurotransmitters, such as dopamine, GABA, histamine, acetylcholine and serotonin. What is interesting, is diet seems to have powerful sway over the state of balance (symbiosis), or imbalance (dysbiosis) of the microbiota, and therefore our mental and brain health'	That Sugar Film
'When your gut microbiome is balanced, you stay healthy, you are in a good mood and you have a lot of energy. When your gut microbiome is out of balance, you are setting yourself up for a host of health issues, including weight gain, diabetes, brain fog, and cancer … The good new[s] is that you can change your gut microbiome. You see, the average lifespan of a bacterium in your microbiome is 20 minutes! So you have the opportunity every time you eat to begin to change the population of your gut microbiome. This is good news because it means that rather than having to subscribe to theories, such as the Paleo diet, which assumes our genes evolve so slowly that we all need to eat like cavemen, we can begin to change our gut microbiome (and thus it's [sic] genes) one meal at a time, and even achieve a healthy gut very quickly'	Dr Northrup

Supplementary Material Table 9: Most popular news articles about MGB shared on Twitter in November and early December 2017.
We made an advanced Twitter search with the phrase: "gut brain microbiota OR bacteria OR micro OR bact OR bug since:2017-11-01" on December 17, 2017. A representative quote for each article is presented in the table. Articles are ordered by decreasing popularity.

'If the research holds up … it may one day be possible to prevent the development of the malformations in susceptible newborns by manipulating their microbiome – perhaps with a simple fecal transplant.'	The New York Times A Baffling Brain Defect Is Linked to Gut Bacteria, Scientists Say 10.06.2017
'these microbes have eons of experience modifying our brains, they are likely to be more precise and subtle than current pharmacological approaches, which could mean fewer side effects.'	The Atlantic When Gut Bacteria Change Brain Function 24.06.2015
'Right now, we don't know enough to justify the claims made for probiotic supplements. The marketing is leagues ahead of the evidence, and we'd do well to view these claims with skepticism.'	Forbes Science Is Showing How Gut Bacteria Affect The Brain, But Don't Bother Taking Probiotics Yet 27.08.2017
'A three-way relationship between the brain, gut and stress hormone cortisol appears to influence how 'messages' are communicated in the body, which may result in autistic symptoms'	Daily Mail Is gut bacteria linked to autism? Pathogens in the stomach alter the brain's development and may increase the risk of condition 25.08.2017
'researchers listed 10 different ways that the microbiome may contribute to the development of Alzheimer's disease, including fungal and bacterial infections in the intestinal tract and increased permeability of the blood-brain barrier.'	Huffington Post Targeting Gut Bacteria May Be The Key To Preventing Alzheimer's 21.02.2017
'Could it be a similar case to that of the human genome – another great hope in predicting disease and personalised preventative medicine, but which becomes more impenetrably complex the more we learn about it?'	The Guardian Is your gut microbiome the key to health and happiness? 6.11.2017
'It's far too soon to know whether the probiotic has any effect, but [a study participant] suspects it might.'	National Public Radio Gut Bacteria Might Guide The Workings Of Our Minds 18.11.2017
'According to influential neurologist from the US, Dr David Perlmutter, there is some good news about the treatment of brain disorders in the future.'	Australian Broadcasting Corporation, All in the Mind The second brain 17.01.2016

'1. Reduce sugar and processed foods … 2. Increase your intake of Omega-3 fats … 3. Eat more tryptophan-containing foods like pork, chicken, seeds and walnuts … 4. Feed your gut bugs'	BBC How food can improve your mental health 22.05.2017
'the findings are enough to determine that "strategies that alter the gut microbiota composition in the elderly," such as developing a good diet and taking probiotics and prebiotics, "reduce inflammaging and promote healthy aging," says Dr Fransen.'	Daily Mail Healthy gut bacteria could help protect you from almost EVERY age-related disease, study finds 2.11.2017

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