A microbial approach to depression

Unravelling the influence of gut microbiota on the onset, development and treatment of depression

Student: Anneke Kastelein

Supervisor: Dr. Bauke Buwalda

Summary

Major depressive disorder affects 350 million people worldwide. Only 11-30% of patients achieve remission on current medication. A fundamentally different approach may dramatically improve treatment outcomes. Research suggests an important role of microbiota in the development of depressive symptoms. Patients with depression show altered microbiome composition, induction of depression in mice induces microbiome alterations. Probiotics have been shown to alter behavior, however data comparing the antidepressants effects of probiotics to antidepressants is scarce. Importantly, the literature on probiotic treatment in animals regarding depressive behavior is exclusively on male subjects. With this project we aim to explore the contribution of gut microbiota on the development of depression and test the therapeutic value of probiotics in depression for both males and females.

We propose to utilize the chronic social defeat model to induce a depressive like phenotype in both male and female mice. Not all mice are equally susceptible to develop a depressive/anxious phenotype, allowing us to study the influence of microbiota in relation to susceptibility to stress. We will investigate the effect of chronic social defeat on microbiota composition, and look at the effects of probiotic treatment on depressive like behavior and microbiota composition. By taking regular fecal samples before and after 10 days of social defeat, and throughout probiotic and antidepressant treatment, we will be able to correlate microbiota to susceptibility and treatment resistance. To confirm the relation between stress susceptibility and microbiota composition we will perform fecal transplants, inserting fecal samples of susceptible mice into non-susceptible mice and vice versa, and look at the effects on development of a depressive phenotype. Additionally we will investigate the role of serotonin in gut-brain communication.

Elucidating the role of microbiota in the onset, development and treatment of depressive symptoms as proposed is a necessary and fundamental step towards probiotic therapy for depression.

Word count: 300

Background

Major depressive disorder (MDD) is one of the major contributors to the global burden of disease. Affecting 350 million people nowadays worldwide (WHO, 2012), the illness is estimated to be among the third worldwide causes of disease burden in 2030 (Mathers & Loncar, 2006). Depression has a lifetime prevalence of 16% (Kessler et al., 2003), and is characterized by a depressed mood, anhedonia, low energy or fatigue, disturbances in sleep and appetite, pessimism, feelings of guilt, low self-esteem and dysregulation of bodyweight (Wong & Licinio, 2001). In addition to its high prevalence, depression is often relapsing and chronic (Mondimore et al., 2006). Although initially 60% of patients improve on given antidepressant medication, just 11-30% achieve remission (Rush et al., 2008). Of those not responding to initial antidepressant therapy, 30-50% remain treatment resistant over time (Mrazek et al., 2014). Additionally, even when medication works well, side effects result in a high noncompliance rate (7-44%) among patients (Khawam, Laurencic & Malone, 2006). Antidepressants have been based on the same mechanisms for over 50 years (Skolnick, Popik & Trullas, 2009). A fundamentally different approach is urgently needed, as it may dramatically improve treatment outcomes in depression.

Recently the gut-brain axis in relation to depression has become of major interest. The gastro-intestinal tract hosts about 100 trillion bacteria, most of which reside symbiotically in the lower intestine (Steven et al., 2006). The microbiota-brain-gut axis is a complex network of communication between the gut, microbiota, and the brain. Even though the field is still in its early stages, research strongly suggests an important role of microbiota in development of depressive symptoms (Dash et al., 2014)(Zhou & Foster, 2015). Two recent studies comparing patients diagnosed with depressive disorder to healthy controls found several significant correlations between depression and microbiota (Naseribafrouei et al., 2014)(Jiang et al, 2015). Induction of chronic depression in C57Bl/6 mice resulted in alterations in the microbiota associated with elevated corticotropin-releasing hormone (CRH) expression, serotonin levels, and colon motility (Park et al., 2013). Chronic exposure to social stress has been shown to change the bacterial community profile (Galley et al., 2014).

Thus, studies suggest a causal relation between microbiota and behavior. Supplementation of probiotics, live microbes that are associated with a beneficial effect, has been used to further study the effect of microbiota on behavior. In healthy volunteers, daily intake of probiotics has shown to improve mood (Benton, Williams & Brown, 2007) as well as having beneficial effects on anxiety and depressive measures and reduced levels of the stress hormone cortisol (Messaoudi et al., 2011). Probiotic treatment reduced depressive and anxiety behaviors in healthy and anxious mice and reduced stress induced corticosterone levels (Savignac et al., 2014) (Bravo et al., 2011). Additionally probiotic treatment has shown to attenuate HPA-axis response and reduce chronic-stress induces abnormal brain plasticity and reduction in neurogenesis (Ait-Belgnaoui et al., 2014). One study has been reported (Desbonnet et al., 2010) showing an antidepressant effect in male rats exposed to maternal separation. However, the researchers only took one behavioral test in account, making it difficult to determine depressive behavior and did not report the effect on microbiome.

It is not clear how microbiota are able to influence mental health, but animal studies have suggested a modulating role for bacteria in the hypothalamic-pituitary-adrenal (HPA) axis response (Sudo et al., 2004). Under stress the hypothalamus secretes CRH, which stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH), triggering the adrenal gland to release glucocorticoids, cortisol in humans and corticosterone in rodents. Maladaptive responses to stress have been implicated in the onset and exacerbation of depression in vulnerable individuals(Bartolomucci & Leopardi, 2009; Pittenger & Duman, 2008). A dysregulation of the HPA axis is a well-known manifestation of depression (Bravo et al., 2009) However, it remains unknown what makes one particular vulnerable to depression in response to stress. Interestingly, in mice, the absence of gastrointestinal microbes resulted in a higher HPA axis stress response (Sudo et al., 2004). This exaggerated response could be rescued by ingestion of commensal *Bificobacterium infantis*, whereas enteropathogenic *Escherichia coli* enhanced the response to stress (Sudo et al., 2004). Hence, microbes might determine ones response to stress.

Importantly, women experience major depression at about twice the rate of men (Holden, 2005; Marcus et al., 2006;Griogoriafis & Robinso , 2007) and respond differentially to different types of antidepressant treatment (Young et al., 2009). Similarly, female animal models respond differently to antidepressant treatment than males (Pitychoutis et al., 2012; Carrier & Kabbaj, 2013; Franceschelli et al., 2015). The neurobiological differences underlying these differences are a neglected area of research, and **current treatments of depression are based almost exclusively on research in male subjects** (Beery & Zucker, 2011). Moreover, research has shown gut **microbiome composition is influenced by sex** (Bolnick et al., 2014; Tennoune et al., 2015).

For the proposed project we will use an animal model of depression. Models relying on an animals' depression like response to chronic stress are often used, as stress plays a fundamental role in the onset of depression in vulnerable individuals (Pittenger & Duman, 2008). We have shown that the chronic social defeat stress model in males induces dysregulation of bodyweight, shows reduced sucrose intake mimicking anhedonia, and increased immobility during a forced swim test thought to be analogous to the despair seen in MDD. Additionally social defeat induces an anxious phenotype. Recently, the same model has been validates in females (Ver Hoeve et al., 2013). Using the same model **allows us to compare male and female** responses. Importantly, not all C57Bl/6 mice are equally susceptible to develop a depressive/anxious phenotype (Krishnan et al., 2007; Kumar et al., 2014). This variability makes social defeat **a powerful and unique tool** to study the influence of microbiota and susceptibility to stress.

Innovation

As reviewed above, probiotics appear a promising treatment option for depression. However, despite the growing interest in brain-gut communication and its role in the pathogenesis of depression, **studies comparing the antidepressants effects of probiotics to antidepressants in depression are scarce**. Moreover, the **effect of probiotic treatment on gut microbiota** composition in depression not known, and the mechanisms by which bacteria of the gut can influence behavior are unclear. With this project we aim to explore the contribution of gut microbiota on the development of depression and test the therapeutic value of probiotics in depression. We will investigate the effect of probiotics on the microbiome, evaluating the **relation between susceptibility and microbiome composition**. The literature on **probiotic treatment in animals regarding depressive behavior is exclusively on male subjects**, therefore it is highly interesting and necessary to research this in female subjects. We will run both male and female cohorts of social defeat, allowing us to compare results of cohorts.

Approach

We intent to test the following aims:

1. Determine the differences in microbiota following chronic social stress exposure

We will establish a social defeat stress model of depression in **both male and female mice** and examine the effect of chronic social defeat on microbiota composition. We will take daily body weight and food intake measurements and collect fecal samples prior and after 10 days of social defeat. We will perform 16s rRNA gene to characterize fecal microbiota. Although *Lactobacilli* and *Bifidobacteria* have been shown to contribute to the interplay of microbiota, stress and depressive behavior, it is likely other bacterial species have an influence (Galley & Bailey, 2014). Therefor we will assess microbiota composition in order to decide which bacteria to include in the probiotic treatment of aim 2. After social defeat we will run a battery of behavioral tests including a social avoidance test, sucrose intake test, elevated plus maze test, open field test, forced swim test and restraint stress test to asses depressive phenotype. During the last test blood samples will be collected prior, during and after recovery of the test for corticosterone analysis. After behavioral testing we will once more collect and analyze fecal samples and correlate this to phenotype. **These correlations will provide us with a reliable biomarker for depression.** Animals will be sacrificed by rapid decapitation. Brain and gut will be removed, blood will be collected for endocrine assays.

2. Compare the effects of chronic probiotic treatment with citalopram, an antidepressant, on the reversibility of a depressive phenotype

We will run a second cohort of social chronic social defeat in both males and females as described in aim 1. After 10 days of social defeat the mice will be divided in three groups to receive daily oral intake via dilution of either probiotic, citalopram or nothing (placebo) in the drinking water for 28 days. Fecal samples will be collected every 7 days for analysis, allowing us to monitor the effect of treatment over time. After treatment of 28 days we will run the battery of behavioral tests to test for depressive like behavior. As we are taking regular fecal samples we will be able to test the effect of antidepressants on microbiota, the effect of the probiotic on microbiota and **test whether non-responders show no alteration in microbiota.** The day after the last behavioral test mice will be sacrificed by rapid decapitation, after which gut, brain and blood will be collected.

3. Test causality of microbiome on susceptibility depressive like behavior

Once we have identified the microbiome of susceptible animals and non-susceptible animals, we will transplant fecal samples of susceptible animals in non-susceptible animals and vice-versa. Resident intestinal microbes will

be suppressed with antibiotics before being treated with fresh fecal contents every other day for a week. After treatment fecal samples will be collected every 7 days for analysis. After 14 days we will run 10 days of social defeat followed by the battery of behavioral tests to test for depressive like behavior. **Fecal transplantation allows us to identify the causal relation between microbiota and susceptibility to depression**. The day after the last behavioral test mice will be sacrificed by rapid decapitation, after which gut, brain and blood will be collected.

4. Identify the mechanisms underlying the influence of gut microbiota on behavior.

Preliminary studies suggest an antidepressant effect of probiotics, however, **how microbes are able to affect behavior is not clear.** We propose serotonin metabolism as potential pathway. Serotonin plays a significant role in brain-gut communication and is recognized as a major biological substrate in the pathogenesis of depression (Crowell & Wessinger, 2007). Several bacteria are known to produce and release serotonin and its precursor tryptophan (Borre et al., 2014), accordingly, germ free mice which show diminished monoaminergic activity (Sudo, 2006). Moreover, serotonin has been shown to modulate HPA-axis activity (Heisler et al., 2007).We will measure serotonin and its metabolite 5-HIAA in the striatum, prefrontal cortex, hypothalamus and amygdala. Blood plasma will be analyzed for tryptophan, L-kynurenine and kynurenic acid. Additionally we will analyze the hypothalamus for CRH and vasopressin by mRNA isolation, c-DNA reverse transcription and real-time PCR.

5. Evaluate the differences in microbiota and probiotic treatment between males and females

We will be using the same stress paradigm for both the males and females. As a result we are able to compare our results, enabling us to **fully elucidate the gender differences in microbiota composition, response to probiotic treatment and susceptibility to depression**. This will allow future research to personalize probiotic treatment to females, rather than only males as is currently the case.

Impact of research

Further investigation of the antidepressant effects of probiotics is the first step towards fundamentally new therapy for depression. We expect to elucidate the differences between male and female subject, thereby optimizing treatment for both sexes. By correlating microbiome to susceptibility and testing this by fecal transplantation we will develop a biomarker distinguishing susceptible animals tot non-susceptible animals. Moreover, we will be able to predict treatment outcome. By elucidating the underlying mechanisms we will take the next step in understanding the brain-gut axis and its relation to depression.

Timeline

Experiments	Timing
Request DEC approval. Set-up housing animals, order animals, order equipment, train resident mice to be aggressive .	1-4 months
Assessing the effects of chronic social defeat on the microbiota. Design probiotic treatment for aim 2.	1-8 months (**research paper 1**)
Assessing the effect of probiotic treatment vs antidepressant treatment on behavior	9-15 months (**research paper 1**)
Assessing the effect of fecal transplantation on stress susceptibility	15-21 months (**research paper 2**)
Assessing the effect of probiotic treatment vs antidepressant treatment on the HPA axis and serotonergic system.	21-27 months (**research paper 2**)
Specifically analyze the differences between male and female mice	27-30 months (**research paper 3**)
Finishing thesis and experiments	30-36 months **(thesis)**

Word count (excluding references): 1931

References

Ait-Belgnaoui, A., A. Colom, V. Braniste, L. Ramalho, A. Marrot, C. Cartier, E. Houdeau, V. Theodorou, and T. Tompkins. "Probiotic gut effect prevents the chronic psychological stress-induced brain activity abnormality in mice."*Neurogastroenterology & Motility* 26, no. 4 (2014): 510-520.A

Almahameed, Amjad. "Peripheral arterial disease: recognition and medical management." *Cleveland Clinic journal of medicine* 73, no. 7 (2006): 621.

Bailey, Michael T., Scot E. Dowd, Jeffrey D. Galley, Amy R. Hufnagle, Rebecca G. Allen, and Mark Lyte. "Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation." *Brain, behavior, and immunity* 25, no. 3 (2011): 397-407.

Bailey, Michael T., Scot E. Dowd, Nicola MA Parry, Jeffrey D. Galley, David B. Schauer, and Mark Lyte. "Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium." *Infection and immunity* 78, no. 4 (2010): 1509-1519.

Bangsgaard Bendtsen KM, Krych L, Sørensen DB, Pang W, Nielsen DS, Josefsen K, et al. (2012) Gut Microbiota Composition Is Correlated to Grid Floor Induced Stress and Behavior in the BALB/c Mouse. PLoS ONE 7(10): e46231. doi:10.1371/journal.pone.0046231

Bartolomucci, Alessandro, and Rosario Leopardi. "Stress and depression: preclinical research and clinical implications." *PloS one* 4, no. 1 (2009): 1-5.

Beery, Annaliese K., and Irving Zucker. "Sex bias in neuroscience and biomedical research." *Neuroscience & Biobehavioral Reviews* 35, no. 3 (2011): 565-572.

Bercik, Premysl, and Stephen M. Collins. "The Effects of Inflammation, Infection and Antibiotics on the Microbiota-Gut-Brain Axis." In *Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease*, pp. 279-289. Springer New York, 2014.

Berton, Olivier, Colleen A. McClung, Ralph J. DiLeone, Vaishnav Krishnan, William Renthal, Scott J. Russo, Danielle Graham et al. "Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress." *Science*311, no. 5762 (2006): 864-868.

Bolnick, Daniel I., Lisa K. Snowberg, Philipp E. Hirsch, Christian L. Lauber, Brian Parks, Aldons J. Lusis, Rob Knight, J. Gregory Caporaso, and Richard Svanbäck. "Individual diet has sex-dependent effects on vertebrate gut microbiota." *Nature communications* 5 (2014).

Borre, Yuliya E., Rachel D. Moloney, Gerard Clarke, Timothy G. Dinan, and John F. Cryan. "The impact of microbiota on brain and behavior: mechanisms & therapeutic potential." In *Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease*, pp. 373-403. Springer New York, 2014.

Bravo, Javier A., Paul Forsythe, Marianne V. Chew, Emily Escaravage, Hélène M. Savignac, Timothy G. Dinan, John Bienenstock, and John F. Cryan. "Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve." *Proceedings of the National Academy of Sciences* 108, no. 38 (2011): 16050-16055.

Carrier, Nicole, and Mohamed Kabbaj. "Sex differences in the antidepressant-like effects of ketamine." *Neuropharmacology* 70 (2013): 27-34.

Crowell, Michael D., and Sarah B. Wessinger. "5-HT and the brain-gut axis: opportunities for pharmacologic intervention." (2007): 761-765.

Dash, Sarah, Gerard Clarke, Michael Berk, and Felice N. Jacka. "The gut microbiome and diet in psychiatry: focus on depression." *Current opinion in psychiatry* 28, no. 1 (2015): 1-6.

Desbonnet, Lieve, Lillian Garrett, Gerard Clarke, John Bienenstock, and Timothy G. Dinan. "The probiotic Bifidobacteria infantis: an assessment of potential antidepressant properties in the rat." *Journal of psychiatric research*43, no. 2 (2008): 164-174.

Desbonnet, L., Lillian Garrett, Gerard Clarke, B. Kiely, J. F. Cryan, and T. G. Dinan. "Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression." *Neuroscience* 170, no. 4 (2010): 1179-1188.

Fodor, Anthony. "Utilizing "omics" tools to study the complex gut ecosystem." In *Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease*, pp. 25-38. Springer New York, 2014.

Fond, Guillaume, Anderson Loundou, Nora Hamdani, Wahid Boukouaci, Aroldo Dargel, José Oliveira, Matthieu Roger, Ryad Tamouza, Marion Leboyer, and Laurent Boyer. "Anxiety and depression comorbidities in irritable bowel syndrome (IBS): a systematic review and meta-analysis." *European archives of psychiatry and clinical neuroscience* 264, no. 8 (2014): 651-660.

Franceschelli, A., J. Sens, S. Herchick, C. Thelen, and P. M. Pitychoutis. "Sex differences in the rapid and the sustained antidepressant-like effects of ketamine in stress-naïve and "depressed" mice exposed to chronic mild stress." *Neuroscience* 290 (2015): 49-60.

Galley, Jeffrey D., and Michael T. Bailey. "Impact of stressor exposure on the interplay between commensal microbiota and host inflammation." *Gut microbes*5, no. 3 (2014): 390-396.

Gill, Steven R., Mihai Pop, Robert T. DeBoy, Paul B. Eckburg, Peter J. Turnbaugh, Buck S. Samuel, Jeffrey I. Gordon, David A. Relman, Claire M. Fraser-Liggett, and Karen E. Nelson. "Metagenomic analysis of the human distal gut microbiome." *science* 312, no. 5778 (2006): 1355-1359.

Glaser, R., & Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction: implications for health. *Nature Reviews Immunology*, *5*(3), 243-251.

Grigoriadis, Sophie, and Gail Erlick Robinson. "Gender issues in depression."*Annals of Clinical Psychiatry* 19, no. 4 (2007): 247-255.

Heisler, Lora K., Nina Pronchuk, Katsunori Nonogaki, Ligang Zhou, Jacob Raber, Loraine Tung, Giles SH Yeo et al. "Serotonin activates the hypothalamic–pituitary–adrenal axis via serotonin 2C receptor stimulation." *The Journal of neuroscience* 27, no. 26 (2007): 6956-6964.

Heisler, Lora K., Nina Pronchuk, Katsunori Nonogaki, Ligang Zhou, Jacob Raber, Loraine Tung, Giles SH Yeo et al. "Serotonin activates the hypothalamic–pituitary–adrenal axis via serotonin 2C receptor stimulation." *The Journal of neuroscience* 27, no. 26 (2007): 6956-6964.

Heisler, Lora K., Nina Pronchuk, Katsunori Nonogaki, Ligang Zhou, Jacob Raber, Loraine Tung, Giles SH Yeo et al. "Serotonin activates the hypothalamic–pituitary–adrenal axis via serotonin 2C receptor stimulation." *The Journal of neuroscience* 27, no. 26 (2007): 6956-6964.

Holden, Constance. "Sex and the suffering brain." *Science* 308, no. 5728 (2005): 1574-1574.

Jeffery, Ian B., Paul W. O'Toole, Lena Öhman, Marcus J. Claesson, Jennifer Deane, Eamonn MM Quigley, and Magnus Simrén. "An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota."*Gut* 61, no. 7 (2012): 997-1006.

Jiang, Haiyin, Zongxin Ling, Yonghua Zhang, Hongjin Mao, Zhanping Ma, Yan Yin, Weihong Wang et al. "Altered fecal microbiota composition in patients with major depressive disorder." *Brain, behavior, and immunity* (2015).

Kessler, Ronald C., Patricia Berglund, Olga Demler, Robert Jin, Doreen Koretz, Kathleen R. Merikangas, A. John Rush, Ellen E. Walters, and Philip S. Wang. "The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R)." *Jama* 289, no. 23 (2003): 3095-3105.

Khawam, E. A., G. Laurencic, and D. A. Malone Jr. "Side effects of antidepressants: an overview." *Cleveland Clinic journal of medicine* 73, no. 4 (2006): 351.

Krishnan, Vaishnav, Ming-Hu Han, Danielle L. Graham, Olivier Berton, William Renthal, Scott J. Russo, Quincey LaPlant et al. "Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions." *Cell* 131, no. 2 (2007): 391-404..

Kumar, Sunil, Rainbo Hultman, Dalton Hughes, Nadine Michel, Brittany M. Katz, and Kafui Dzirasa. "Prefrontal cortex reactivity underlies trait vulnerability to chronic social defeat stress." *Nature communications* 5 (2014).

Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughoutthe lifespan on the brain, behaviour and cognition. Nature Rev Neurosci2009;10:434–45.

Lyte, Mark, Wang Li, Noel Opitz, Ronald PA Gaykema, and Lisa E. Goehler. "Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia Citrobacter rodentium." *Physiology & behavior* 89, no. 3 (2006): 350-357.

Marcus, Sheila M., Elizabeth A. Young, Kevin B. Kerber, Susan Kornstein, Amy H. Farabaugh, Jeff Mitchell, Stephen R. Wisniewski, G. K. Balasubramani, Madhukar H. Trivedi, and A. John Rush. "Gender differences in depression: findings from the STAR* D study." *Journal of affective disorders*87, no. 2 (2005): 141-150.

Mathers, Colin D., and Dejan Loncar. "Projections of global mortality and burden of disease from 2002 to 2030." *Plos med* 3, no. 11 (2006): e442.

Messaoudi, Michaël, Robert Lalonde, Nicolas Violle, Hervé Javelot, Didier Desor, Amine Nejdi, Jean-François Bisson et al. "Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects." *British Journal of Nutrition* 105, no. 05 (2011): 755-764.

Mondimore, Francis M., Peter P. Zandi, Dean F. MacKinnon, Melvin G. McInnis, Erin B. Miller, Raymond P. Crowe, William A. Scheftner et al. "Familial aggregation of illness chronicity in recurrent, early-onset major depression pedigrees." *The American journal of psychiatry* 163, no. 9 (2006).

Mrazek, David A., John C. Hornberger, C. Anthony Altar, and Irina Degtiar. "A review of the clinical, economic, and societal burden of treatment-resistant depression: 1996–2013." *Psychiatric services* (2014).

Naseribafrouei, A., K. Hestad, E. Avershina, M. Sekelja, A. Linløkken, R. Wilson, and K. Rudi. "Correlation between the human fecal microbiota and depression." *Neurogastroenterology & Motility* 26, no. 8 (2014): 1155-1162.

Park, A. J., J. Collins, P. A. Blennerhassett, J. E. Ghia, E. F. Verdu, P. Bercik, and S. M. Collins. "Altered colonic function and microbiota profile in a mouse model of chronic depression." *Neurogastroenterology & Motility* 25, no. 9 (2013): 733-e575.

Pittenger, Christopher, and Ronald S. Duman. "Stress, depression, and neuroplasticity: a convergence of mechanisms." *Neuropsychopharmacology*33, no. 1 (2008): 88-109.

Pitychoutis, P. M., C. Dalla, A. C. Sideris, P. A. Tsonis, and Z. Papadopoulou-Daifoti. "5-HT 1A, 5-HT 2A, and 5-HT 2C receptor mRNA modulation by antidepressant treatment in the chronic mild stress model of depression: sex differences exposed." *Neuroscience* 210 (2012): 152-167.

Rush, A. John, Stephen R. Wisniewski, Diane Warden, James F. Luther, Lori L. Davis, Maurizio Fava, Andrew A. Nierenberg, and Madhukar H. Trivedi. "Selecting among second-step antidepressant medication monotherapies: predictive value of clinical, demographic, or first-step treatment features."*Archives of General Psychiatry* 65, no. 8 (2008): 870-880.

Savignac, H. M., B. Kiely, T. G. Dinan, and J. F. Cryan. "Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice." *Neurogastroenterology & Motility* 26, no. 11 (2014): 1615-1627.

Skolnick, Phil, Piotr Popik, and Ramon Trullas. "Glutamate-based antidepressants: 20 years on." *Trends in Pharmacological Sciences* 30, no. 11 (2009): 563-569.

Sudo, Nobuyuki, Yoichi Chida, Yuji Aiba, Junko Sonoda, Naomi Oyama, Xiao-Nian Yu, Chiharu Kubo, and Yasuhiro Koga. "Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice." *The Journal of physiology* 558, no. 1 (2004): 263-275.

Tennoune, Naouel, Romain Legrand, Wassila Ouelaa, Jonathan Breton, Nicolas Lucas, Christine Bole-Feysot, Jean-Claude do Rego, Pierre Déchelotte, and Sergueï O. Fetissov. "Sex-related effects of nutritional supplementation of Escherichia coli: Relevance to eating disorders." *Nutrition* 31, no. 3 (2015): 498-507.

Tillisch, Kirsten, Jennifer Labus, Lisa Kilpatrick, Zhiguo Jiang, Jean Stains, Bahar Ebrat, Denis Guyonnet et al. "Consumption of fermented milk product with probiotic modulates brain activity." *Gastroenterology* 144, no. 7 (2013): 1394-1401.

Ver Hoeve, Elizabeth S., Geralyn Kelly, Sandra Luz, Serena Ghanshani, and Seema Bhatnagar. "Short-term and long-term effects of repeated social defeat during adolescence or adulthood in female rats." *Neuroscience* 249 (2013): 63-73.

Wong, Ma-Li, and Julio Licinio. "Research and treatment approaches to depression." *Nature Reviews Neuroscience* 2, no. 5 (2001): 343-351.

World Health Organisation. " Depression A global health concern" http://www.who.int/mental_health/management/depression/who_paper_depression_wfmh_2012.pdf?ua=1. Accessed 06.08.2015

Young, Elizabeth A., Susan G. Kornstein, Sheila M. Marcus, Anne T. Harvey, Diane Warden, Stephen R. Wisniewski, G. K. Balasubramani, Maurizio Fava, Madhukar H. Trivedi, and A. John Rush. "Sex differences in response to citalopram: a STAR∗ D report." *Journal of psychiatric research* 43, no. 5 (2009): 503-511.

Zhou, Yun, Dan Zhao, Charles G. Winkworth-Smith, Tim J. Foster, Satoru Nirasawa, Eizo Tatsumi, and Yongqiang Cheng. "Effect of a small amount of sodium carbonate on konjac glucomannan-induced changes in wheat starch gel." *Carbohydrate polymers* 116 (2015): 182-188.