Normal gut microbiota modulates brain development and behavior

Rollchellys Diaz Heijitz, Shugui Wang, Farhana Anuar, Yu Quian, Britta Bjorkholm, Annika Samuelsson, Martin L Hibberd, Hans Forssberg, and Sven Pettersson.

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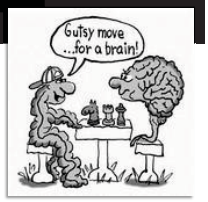

Nischay Shah & Christopher Hwu
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I. The Journal

About PNAS

A. Proceedings of the National Academy of Sciences of the United States of America

B. One of the world's most-cited multidisciplinary scientific serials

C. Established in 1914; Publishes cutting-edge research reports, commentaries, reviews, perspectives, colloquium papers, and actions of the Academy

D. Coverage in PNAS spans the biological, physical, and social sciences

E. PNAS is published weekly in print, and daily online in PNAS Early Edition

F. The PNAS impact factor is 9.737

II. The Institutions

A. Karolinska Institute

1. Founded in 1810.
2. One of Europe's largest and most prestigious medical universities in Stockholm, Sweden.
3. A committee of the institute appoints the laureates for the **Nobel Prize in Physiology & Medicine**.

II. The Institutions

B. Stockholm Brain Institute

1. *Main Focus*: Translation Neuroscience Research
2. Serves as a base for internationally leading and developing research groups from **THREE** Stockholm area universities, Karolinska Institute (KI), Royal Institute of Technology (KTH), and Stockholm University (SU).

II. The Institutions

C. Genome Institute of Singapore

1. Established by the Agency for Science, Technology and Research in 2003.
2. Goal of the institute is to use genomic sciences to improve public health and public prosperity.

III. The People

A. Rochellys Diaz Heijtz, PhD

1. Associate Professor
   a. Department of Neuroscience (Karolinska Institute)
   b. September 2012 – Present
2. Founding Coordinator and Lecturer of the postgraduate course entitled "**Brain Development and Neurodevelopmental Disorders**," Frontier Courses in Neuroscience
3. *Other group member*: **Yu Qian**, PhD student

III. The People

B. Hans Frossberg, PhD

1. Professor of Basal and Clinical Neuroscience at the Department of Woman and Child Health, Karolinska Institute.
2. Director of the Strategic Research Programme in Neuroscience.
3. *Research Interests*: Neurological development and how the brain's control of motor and cognitive functions develops.
4. Conducts both clinical studies in children with cerebral palsy, ADHD, autism and language disorders, and translational research in various animal models.

III. The People

C. Sven Petterson, MD

1. Professor of Host-Microbe Interactions.
   a. Appointed Professor in 2001.
   b. Active in the Department of Microbiology, Tumor and Cell Biology, Karolinska Institute.

III. The People

D. Annika Samuelsson

1. Biomedical Scientist, Petterson Group, Karolinska Institute

E. Britta Björkholm, PhD

1. Responsible for the functional genomic approach to understand how microbes contribute to and tune normal gut homeostasis (cellular microbiology) in Petterson Group

F. Farhana Anuar

1. Postdoctoral Fellow at Karolinska Institute; Past: Research Associate at A*STAR - Agency for Science, Technology and Research


Annika Samuelsson


Farhana Anuar

III. The People

G. Martin L. Hibbered

1. **Senior Investigator**, Genome Institute Singapore.
2. Associate Director, Infectious Diseases.
3. Education:
   a. 1994, King's College, London University: Ph.D. (Medicine).
   b. 1985, Brunel University (West London): B.Sc. (Hons) Applied Biology.

III. The People

H. Shugui Wang

1. Scientist of gut microbiology, Danone Singapore
2. *Past*: Research Fellow at National Cancer Centre, Research Fellow at Genome institute of Singapore
3. *Education*: National University of Singapore (NUS), Sun Yat-Sen University

IV. Background

A. Developmental Programming

1. Its Impacts On Structure And Function Of Organs For The Duration Of Life
2. Systemic Effects On Liver Functions
3. Other Potential Effects?

B. Symbiotic Relationship With Mammals

C. Susceptibility To Internal And External Cues During Perinatal Life

1. Neurological Disorders
   a. Autism
   b. Schizophrenia

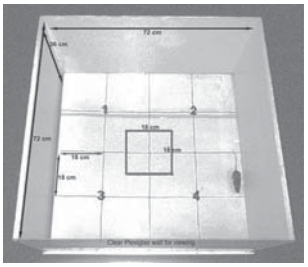
V. Purpose And Hypothesis

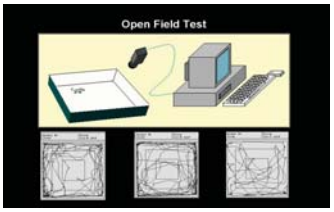
A. Purpose: To see whether the nonpathogenic gut microbiota could affect anxiety like behavior.

B. Hypothesis: "… the normal gut microbiota is an integral part of the external environmental signals that modulate brain development and function."

VI. Methods

A. Open Field Test

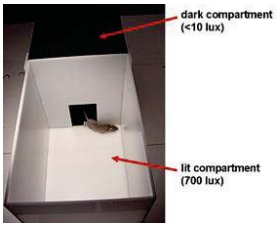




This test is commonly used as qualitative and quantitative measure of general locomotor activity and willingness to explore in rodents.

VI. Methods

B. Light-Dark Box Test



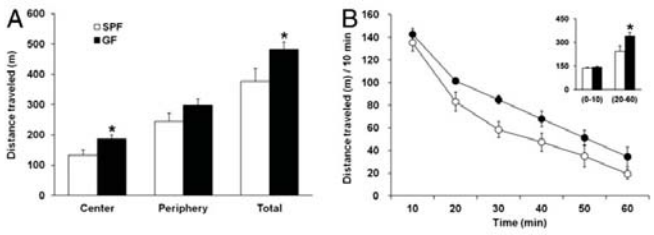
The light/dark box test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, novel environment and light.

VI. Methods

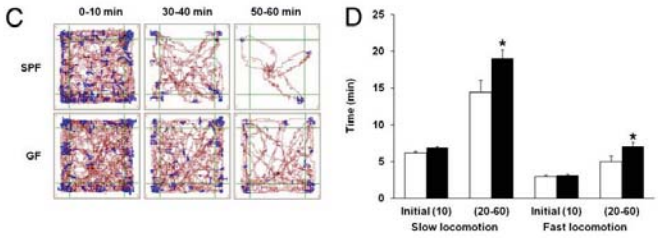
C. Elevated Plus Maze Test

1. The elevated plus maze test is one of the most widely used tests for measuring anxiety-like behavior.
2. The test is based on the natural aversion of mice for open and elevated areas, as well as on their natural spontaneous exploratory behavior in novel environments.

VII. GF mice display increased spontaneous motor activity

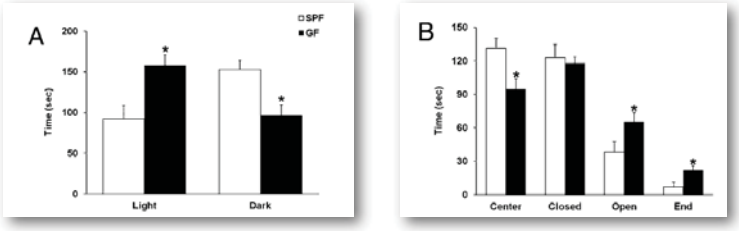


VII. GF mice display increased spontaneous motor activity



VIII. GF mice display reduced anxiety-like behavior

Mice are known for their innate aversion to brightly illuminated area.



IX. Elevated Plus Maze Test

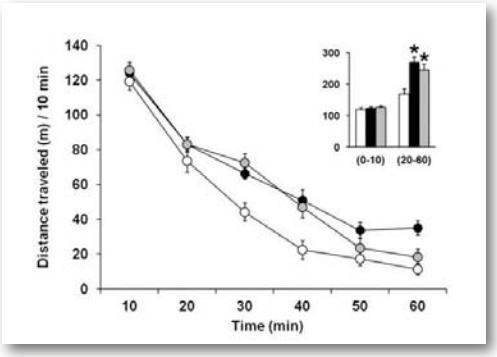
A. Germ Free Mouse

IX. Elevated Plus Maze Test

B. (Specific Pathogen Free) SPF Mouse

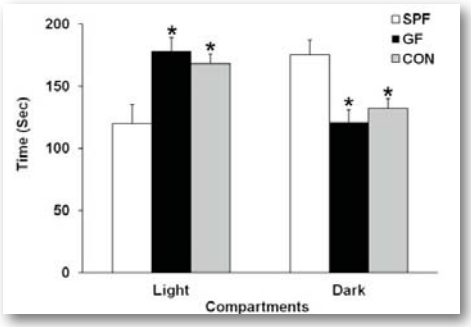
X. Data For CON Mice

A. Open Field Test



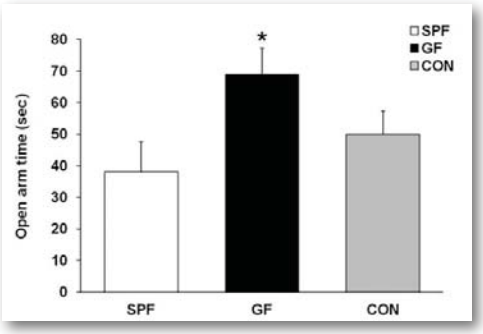
X. Data For CON Mice

B. Light-Dark Box Test

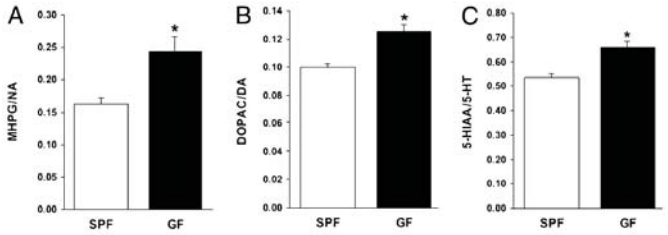


X. Data For CON Mice

C. Elevated Plus Maze Test



XI. GF mice show altered expression of anxiety and synaptic plasticity related genes



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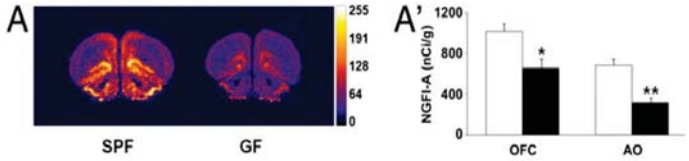

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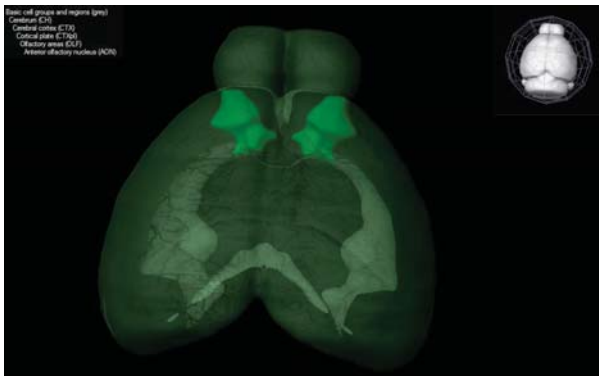

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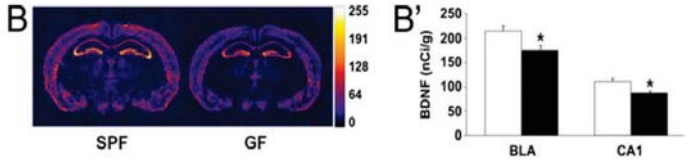

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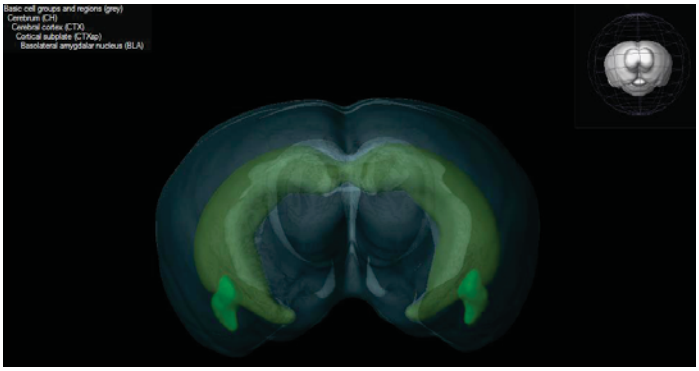

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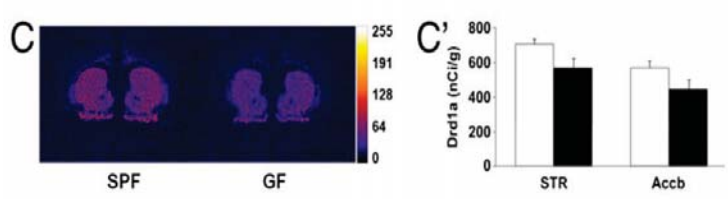

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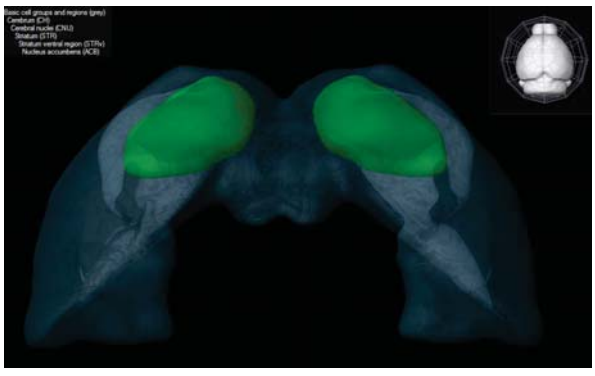

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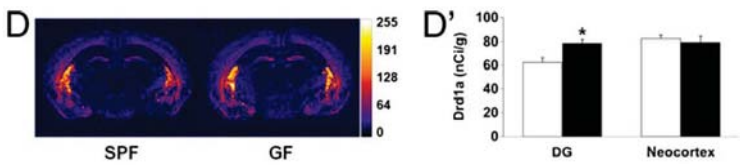

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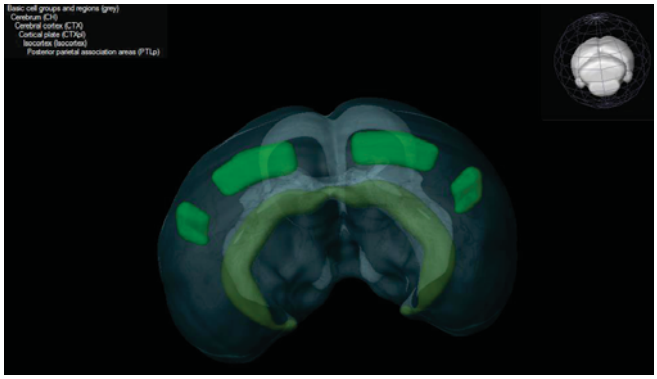
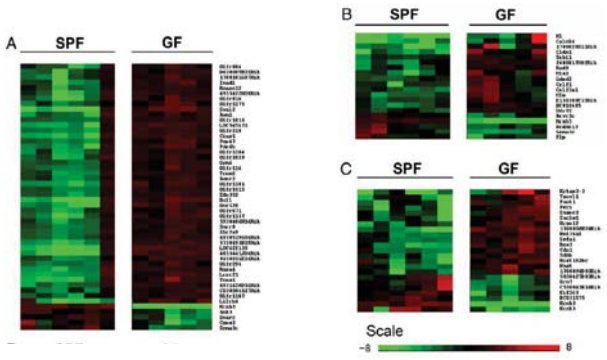
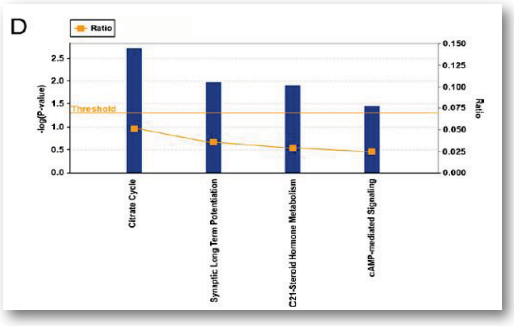


XII. Expression profiling of GF mice and SPF mice brains



XIII. Differentially expressed genes clustered in canonical pathways



XIV. GF mice show higher expression of synaptic related proteins in striatum compared with SPF mice

Table 1. Expression of synaptophysin and PSD-95 in various brain regions of GF, SPF, and CON mice

	Frontal cortex		Striatum		Hippocampus	
Mice	Synaptophysin	PSD-95	Synaptophysin	PSD-95	Synaptophysin	PSD-95
GF	0.989 ± 0.058	1.039 ± 0.085	**1.803 ± 0.037***	**2.171 ± 0.042***	0.982 ± 0.046	1.025 ± 0.136
SPF	1.000 ± 0.062	1.000 ± 0.145	1.000 ± 0.015*	1.000 ± 0.036*	1.000 ± 0.051	1.000 ± 0.075
CON	0.992 ± 0.022	1.011 ± 0.090	1.013 ± 0.028*	1.001 ± 0.063*	0.973 ± 0.039	1.045 ± 0.076

Expression of synaptophysin and PSD-95 in the frontal cortex, striatum, and hippocampus of GF ($n = 4$), SPF ($n = 4$), and CON ($n = 6$) mice were quantified and calculated against their respective actin expression (Fig. 6). The values were then compared against the SPF mice values and expressed as average fold increase. Values are expressed as means ± SEM.
*$P < 0.05$ compared with SPF mice.

XV. Discussion Questions

A. Describe the differences between synaptophysin and PSD-95 protein.
B. How convincing are the results? Can you think of additional biochemical methods and/or experiments that could be factored into this study to produce more informative conclusions?
C. Can you think of at least **FIVE** factors that can produced variation in a core human microbiome?
D. Do you think the overuse of antibiotics affects the brain development of human infants? Explain your reasoning.
E. What is the significance of the data and results gained from this study? What future directions or experiments could be done?

XVI. Conclusions

A. Normal gut microbiota can affect normal brain development and behaviors.
1. ACTH levels, signaling pathways, neurotransmitter turnover, and synaptic-related proteins are affected.
2. Affect motor control and anxiety-like behavior.
3. Synaptophysin and PSD-95 in the striatum are modulated during synaptogenesis.

B. Gut microbiota are able to modulate only if exposed early during postnatal development.
1. Periphery serotonin are carefully monitored and regulated.

C. Mediating the gut-brain communication may be through established neuronal circuits (includes modulation of transmitters).
1. Same pathways that regulates food intake, bone remodeling, and behavioral brain functions.

XVI. Conclusions

D. Usages
1. Gut microbiota can be used to modify expression of risk genes, and alter cognitive functions in patients with gastrointestinal diseases.

E. Can be useful in the study of psychiatric disorders in humans.

XVI. Conclusions

Thoughts, Comments, Questions?

Thank You.