DECONSTRUCTION & ANALYSIS OF STUDY OF MICROBIAL RECONSTITUTION IN SOCIAL & SYNAPTIC DEFICIENT MICE

Amelia Long • Sahar Uppal • Harry Mogg • Caitlin Shepherd • Lia Lloyd

Robin Little • Dec Sheftz • Harsha Shaju

INTRODUCTION

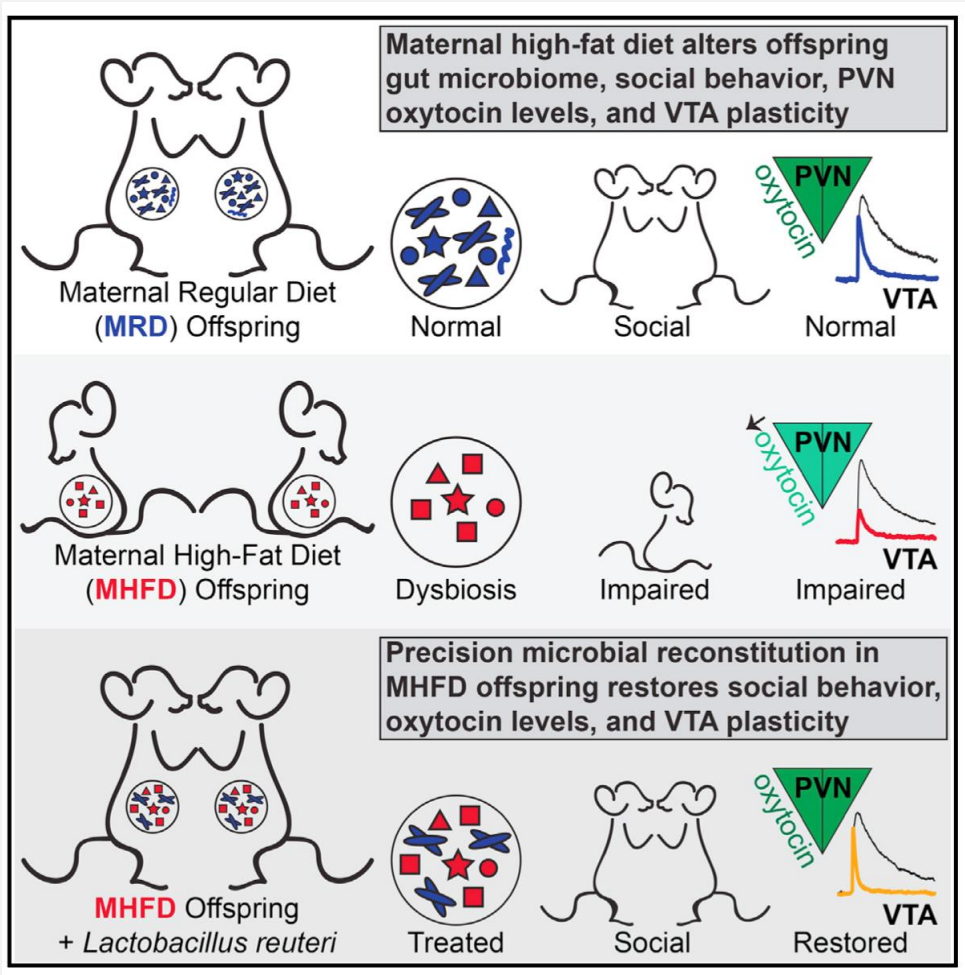
Amelia Long

- This paper discusses a potential link between maternal obesity during pregnancy and an increased risk of neurodevelopmental disorders, such as autism spectrum disorder (ASD) in offspring.

- In this paper to study this link, a maternal high fat diet was given to pregnant mice, to study the offspring. This was compared to mice on a regular diet.

INTRODUCTION

Amelia Long



Highlights of the paper

Amelia Long

- Maternal high-fat diet (MHFD) induces behavioural alterations in offspring.
- MHFD causes alterations in gut microbial ecology in offspring.
- MHFD offspring show deficient synaptic plasticity in the VTA and oxytocin production.
- L. reuteri treatment restores oxytocin levels, VTA plasticity and social behaviours.

Autism Spectrum Disorder

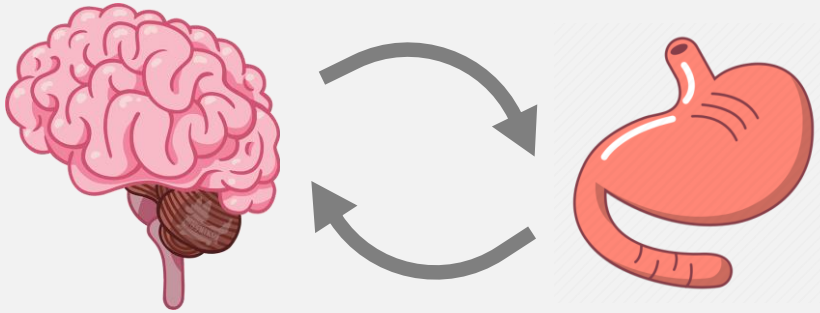
Amelia Long

- ASD is a neurological and developmental disorder.
- Autistic people can find it hard to communicate and interact with other people (1), which is why the social behaviours are looked at.

- ASD is not curable but this paper suggests that it can be preventable, which is problematic for many people who consider autism to be a key part of themselves and not a detriment.

Brain-gut-microbiome axis

Amelia Long



- The link between the brain and the gut is a growing area of study, and more and more studies are supporting the links between the two.
- It is important to look at an organism as a whole
- Studies have shown bidirectional interactions within the brain-gut-microbiome axis.
- Many physiological channels that include neuroendocrine and neuroimmune pathways, as well as the autonomic nervous system. Bacteria can produce neurotransmitter. (3).
- A number of neurologic disorders have been linked with the gut, such as: depression and anxiety, Parkinson's disease, and ASD. (2)

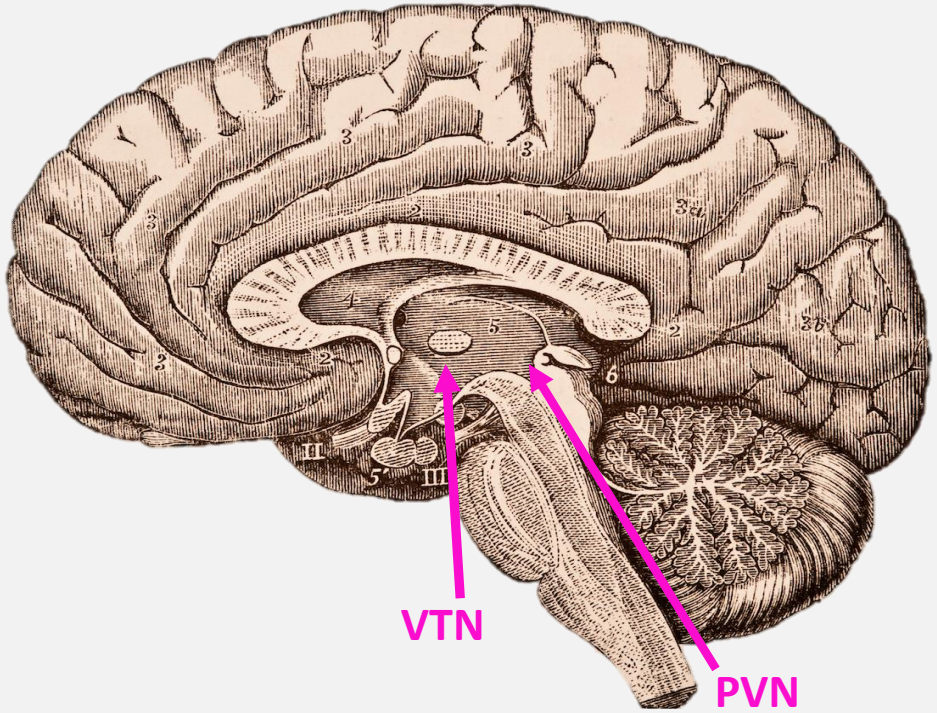
NEUROSCIENCE BACKGROUND

Sahar Uppal

Ventral Tegmental Area (VTA)

Primary role: assessment of need to regulate reward consumption

Located in the midbrain and sends dopaminergic projections to limbic + cortical areas



VTA = dopamine production
PVN = oxytocin production

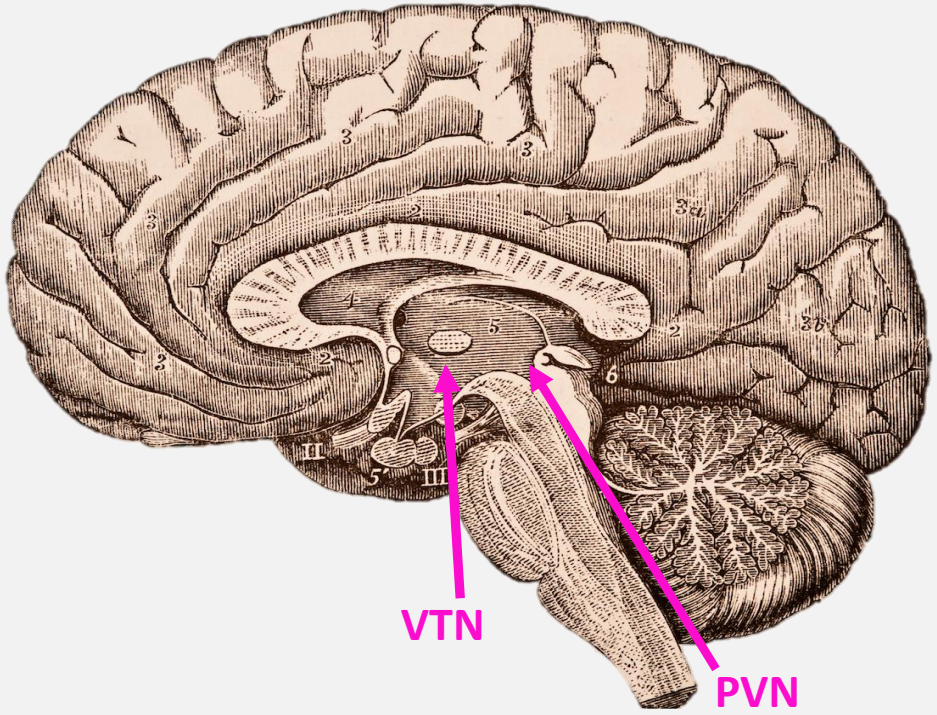
NEUROSCIENCE BACKGROUND

Sahar Uppal

Paraventricular Nucleus (PVN)

Primary role: multifaceted, can help behavioural regulation (also homeostatic regulation)

Located in the hypothalamus, can project to VTA and pituitary gland

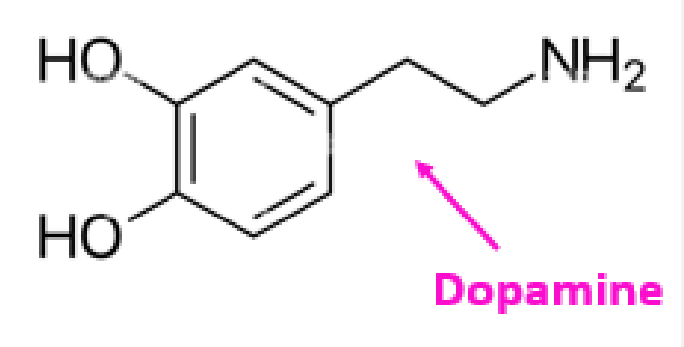


VTA = dopamine production
PVN = oxytocin production

Roles of the PVN and VTA

Sahar Uppal

- VTA: dopamine is a key neurotransmitter in reward-based behaviour
- Reward pathway of VTA: appetite-based behaviour induced by an attractive outcome (a primary reward)

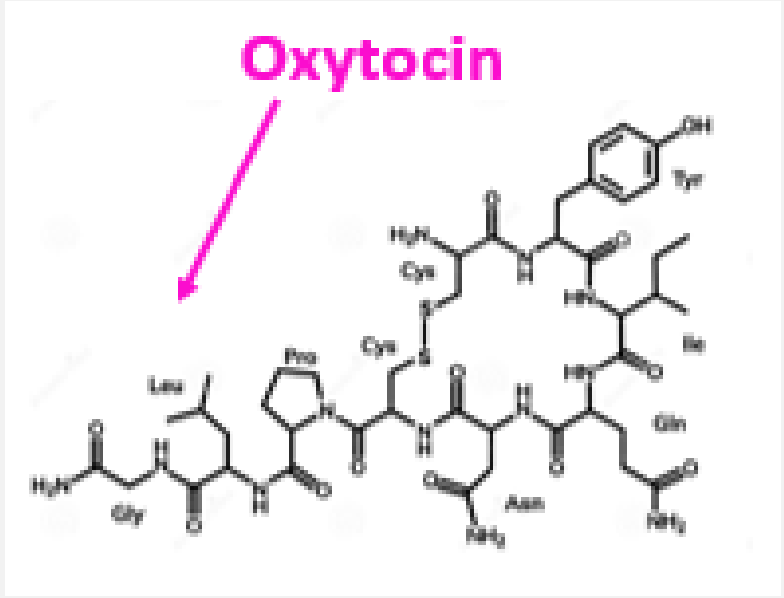


- Primary reward= parental bonding, social contact, eating/drinking
- Promotes your own and your offspring's survival

Roles of the PVN and VTA

Sahar Uppal

- PVN: releases oxytocin to neurons in the VTA.
- Oxytocin regulates pro-social behaviours e.g. trust, communication, bonding

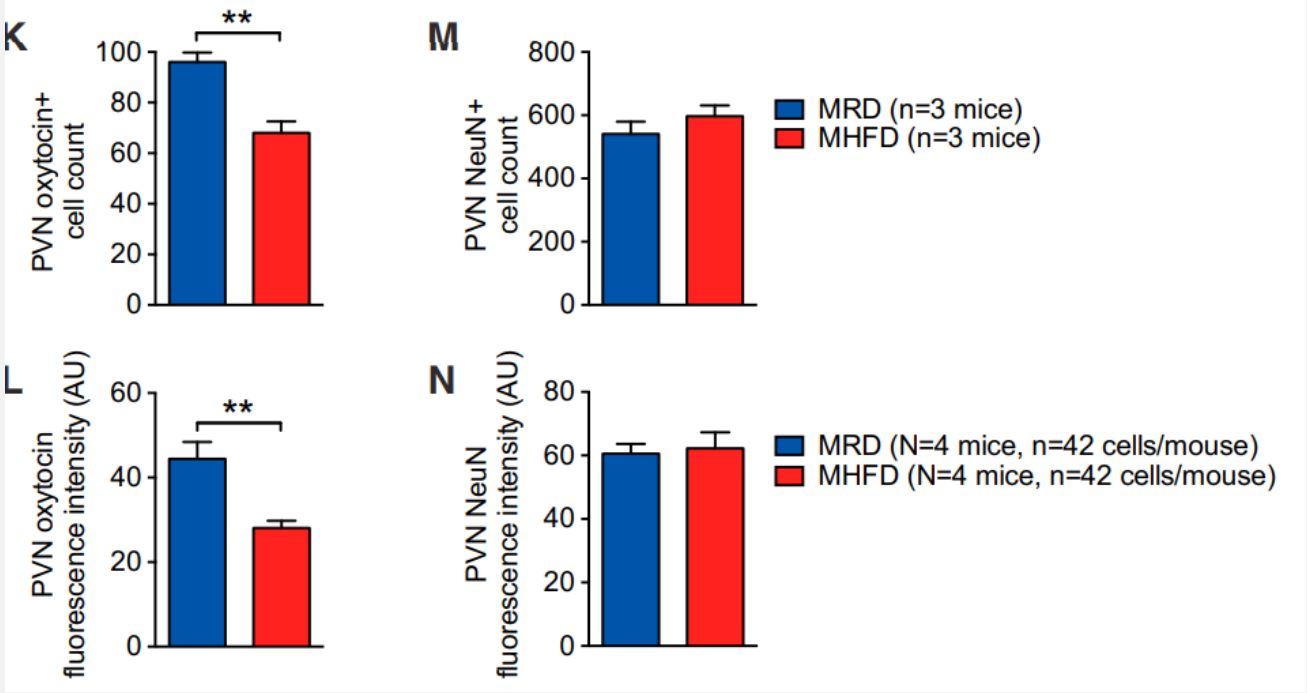


Mesolimbic dopamine reward system (MDRS) is impaired in MHFD offspring.

- MDRS= dopaminergic inputs from VTA innervate brain regions
- Mediates pleasure + reward
- Oxytocin expressing neurons project from the PVN to the VTA + activate neuronal response to social cues
- Blockage of VTA oxytocin receptors prevents social attachment

Sahar Uppal

- Compared the levels of oxytocin expressing cells in the PVN of MRD vs MHFD
- Result: less immunoreactive neurons in MHFD



- Same number of total neurons
- L.Reuteri treatment successfully increases oxytocin levels in MHFD mice

Sahar Uppal

Can direct social interaction induce LTP of synaptic input to VTA of dopaminergic neurons?

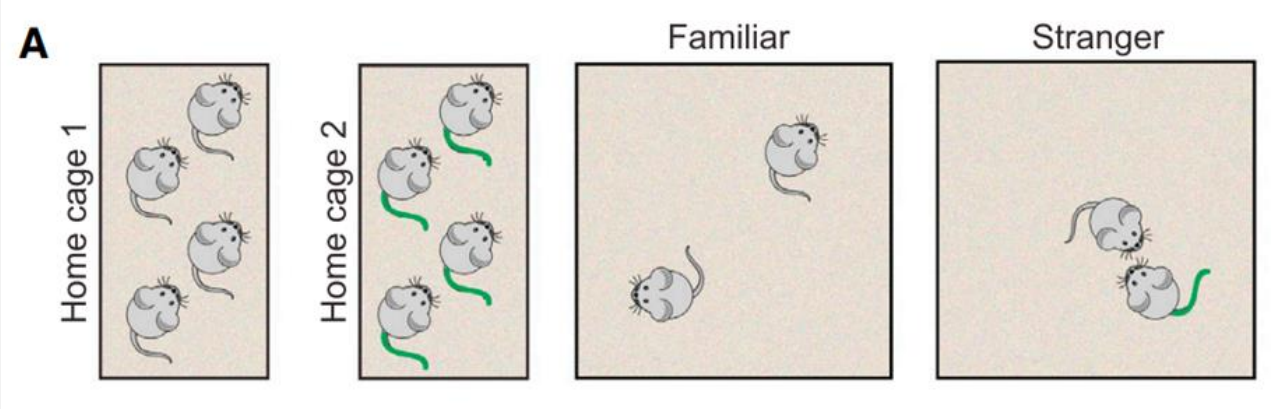
LTP= increase of synaptic strength

Social interaction should induce a long lasting increase in activity of MDRS

Glutamatergic post synaptic currents measured when interacting with a stranger and a familiar mouse

MRD: more time spent with stranger, LTP induced

MHFD: neither of these occurred



Methods

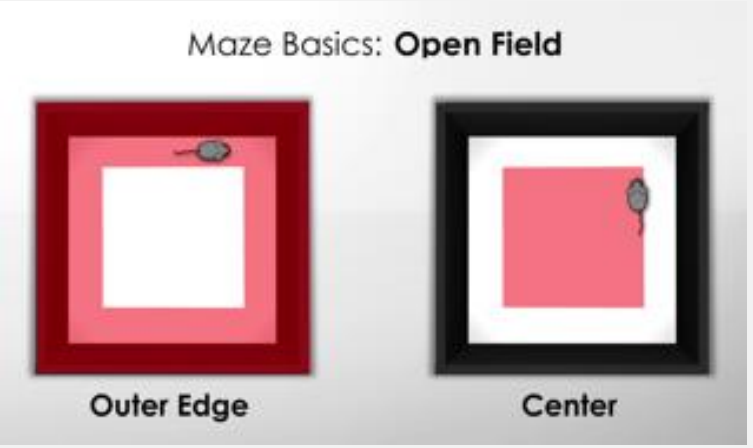
Harry Mogg

Open Field Assay

Rodents display an aversion to brightly lit areas – bright light has been shown to cause damage to their retinas *, they also have a drive to explore.

This test uses these facts to try and measure anxiety levels by recording where the mouse is located within the field

Increased anxiety will result in less activity and staying closer to the edge of the field



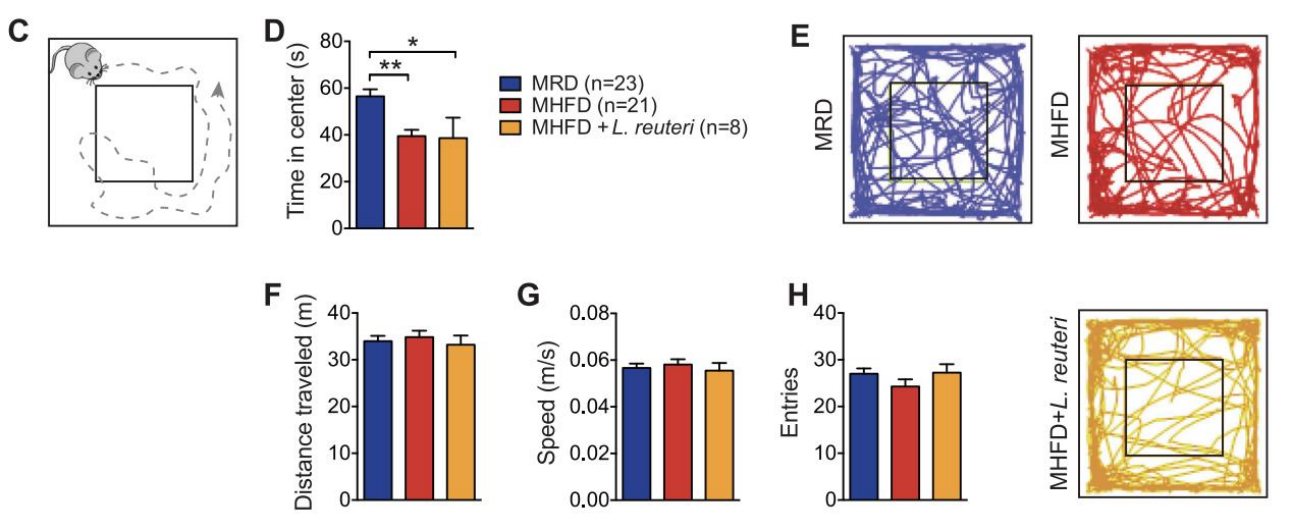
* Castelhano-Carlos MJ, Baumans V. (2009) The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory Animals*.

Methods

Harry Mogg

MHFD did change the amount of time spent in the center

Measures of locomotive activity did not differ between maternal diet or treatment group



(C) Schematic of the open field test; open field activity represents a measure of changes in anxiety-like behaviors.
(D) Compared to MRD, MHFD offspring spent less time exploring the center of the open field arena ($p < 0.05$, $t = 3.61$) and *L. reuteri* treatment did not significantly affect this behavior (MHFD versus MHFD+*L.reuteri*, $p > 0.99$, $t = 0.13$; MRD versus MHFD+*L. reuteri*, $p < 0.05$, $t = 2.79$; $F_{2,49} = 7.82$, $p < 0.01$).
(E) Representative plots of exploratory activity.
(F–H) Measures of locomotor activity during arena exploration did not differ between maternal diet or treatment groups (F, $F_{2,49} = 0.26$, $p = 0.77$; G, $F_{2,49} = 0.25$, $p = 0.78$; H, $F_{2,49} = 1.30$, $p = 0.28$). Plots show mean ± SEM.

Methods

Harry Mogg

Marble Burying Assay

When rodents are put into a cage with marbles, they will burry them, this is positively related to anxiety or obsessive-compulsive disorder

Mice burry the marbles because they show a tendency to dig in natural settings e.g., burrows. *

It can easily be quantified by the amount buried in 20 minutes

Co housing and colonization with microbiota do not reverse the increased marble burying behavior

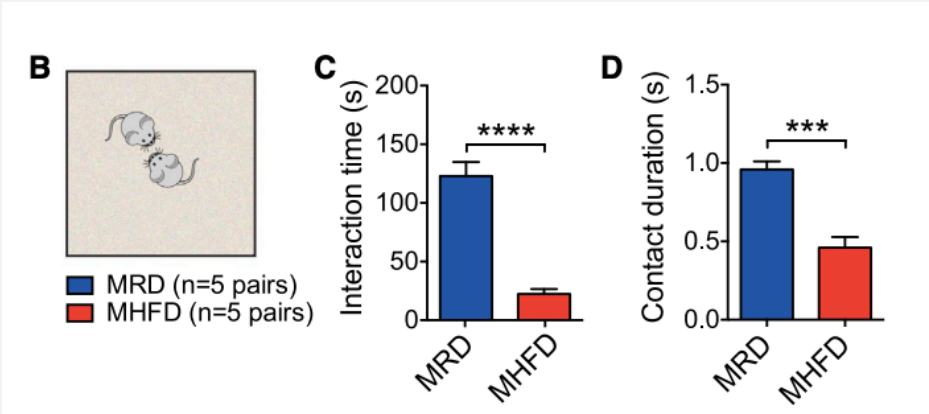
*Angoa-Pérez M, Kane MJ, Briggs DI, Francescutti DM, Kuhn DM. (2013) Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviours in mice.

Methods

Harry Mogg

Reciprocal Social Interaction

- Behaviors are recorded and analyzed to assess social interaction time
- A test mouse is placed with a novel mouse
- Mice with social deficits will show a reduced time of time engaged in social interaction
- Mice must be the same sex and similar sizes to prevent dominance altering the results



Methods

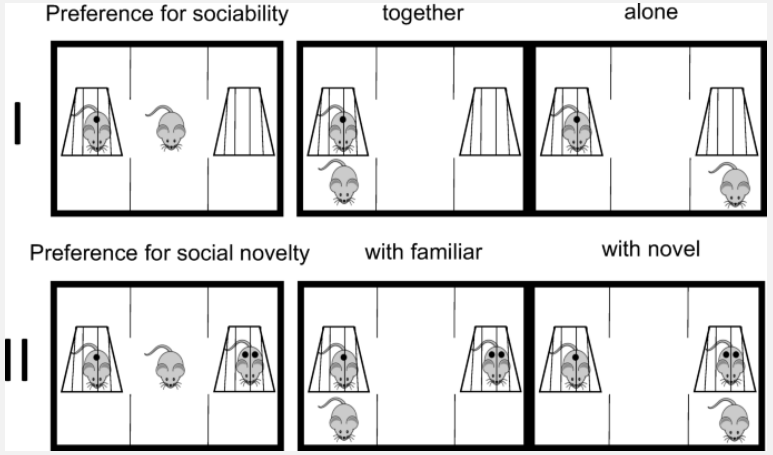
Harry Mogg

Three Chamber Social Interaction

This test tests for symptoms relating to ASD specifically sociability and preference for novel sociability.

Firstly, the mouse is habituated in the empty chamber

There are three sections, one will be filled with a novel mouse the other with a novel object



It should show preference for being social

Then a second mouse is added

Times socializing with both new mice are recorded

Methods

Harry Mogg

Three Chamber Social Interaction

- GF mice were shown to have impaired sociability and preference for social novelty (C and D)
- GF mice colonized with fecal transplant from MRD restored both GF sociability (E and F)
- No difference seen with colonization at 8 weeks

The Three-chamber test has been used to link the Neuregulin-1 (NRG1) gene to schizophrenia*

*O'Tuathaigh CM, Babovic D, O'Sullivan GJ, Clifford JJ, Tighe O, Croke DT, Harvey R, Waddington JL. (2007) Phenotypic characterization of spatial cognition and social behavior in mice with 'knockout' of the schizophrenia risk gene neuregulin 1. Neuroscience.

Methods

Harry Mogg

Review of the assays

The paper uses 4 assays to assess levels of anxiety, compulsive behaviors and disordered social interactions.

The tests can be classed into two classes of test

- Assays for social interactions: Reciprocal social interaction & three chamber social chamber
- Assays for repetitive behavior: Marble burying assay & open field assay

To improve the paper, I would add a communication Assay that assesses

Ultrasonic vocalizations. Mouse pups have been found to use ultrasonic vocalization to find their mother. ASD has a large effect on these behaviors when juvenile and on maternal behaviors.

Results

Caitlin Shepherd

General findings

- MHFD increased maternal weight, time to first litter & frequency of spontaneous abortion
- Decreased litter size
- ASD associated traits shown e.g. repetitive behaviour, anxiety
- These weren't corrected by either co-housing or transplant so indicate other factors

Results

Caitlin Shepherd

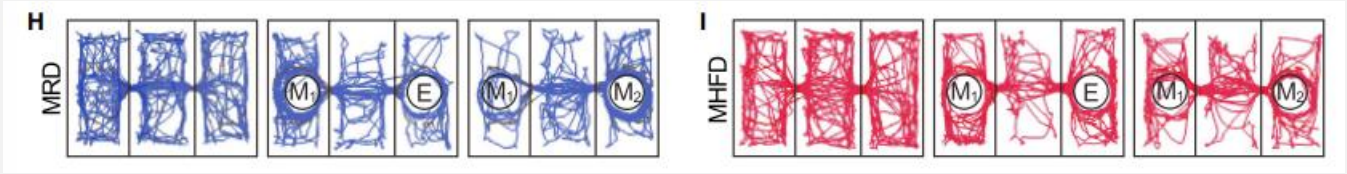
Social behaviour

Reciprocal interactions -> MHFD fewer

Sociability -> Impaired in MHFD

Preference for familiarity -> MHFD no preference

However doesn't demonstrate causality



Results

Caitlin Shepherd

Co-housing

1 MHFD mouse co-housed with 3 MRD mice, compared to controls of 4 MRD mice and 4 MHFD mice.

Exhibited normal reciprocal interactions, sociability & preference following 7-8 weeks co-housing

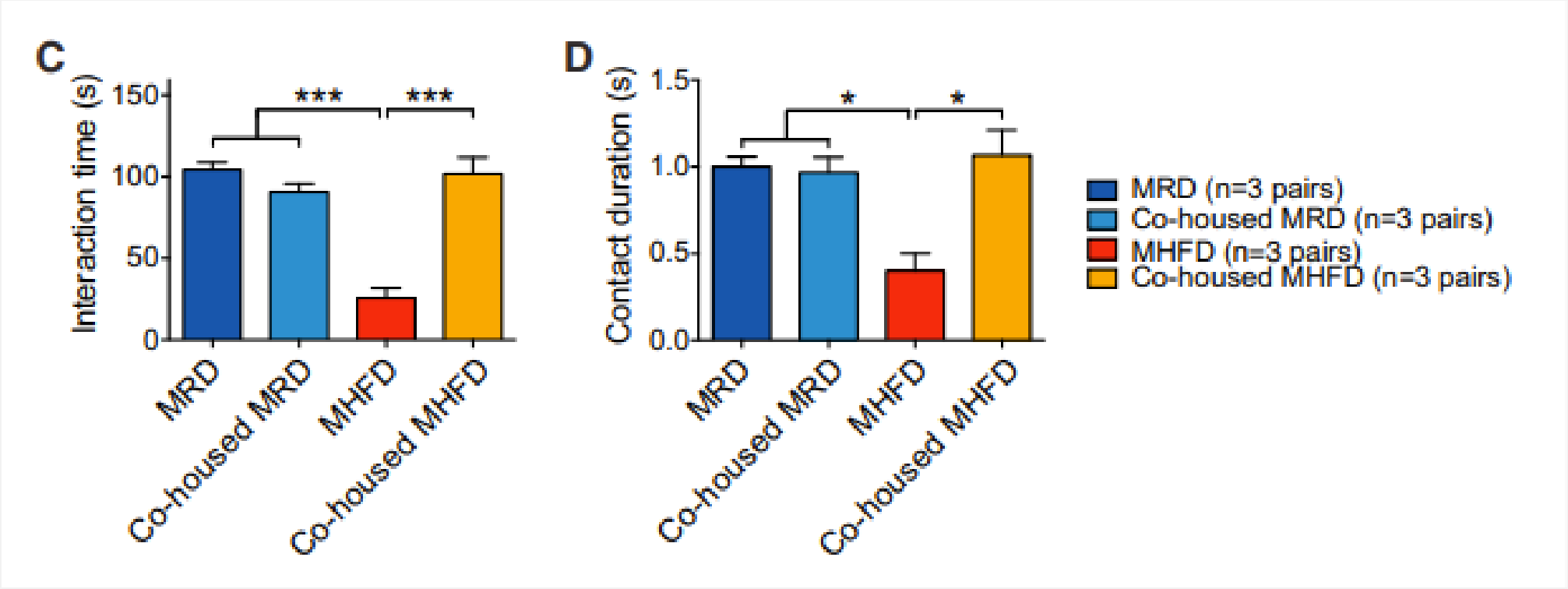
Also caused shift in bacteria species found, more similar to MRD

Suggests specific bacteria required for normal social behaviour

Results

Caitlin Shepherd

Co-housing



Results

Caitlin Shepherd

Colonisation of germ-free mice

Germ-free mice deficient in gut microbiome, exhibited expected abnormal social behaviour

MRD faecal transplant corrected behaviour at 4 weeks but not 8

MHFD didn't correct at either

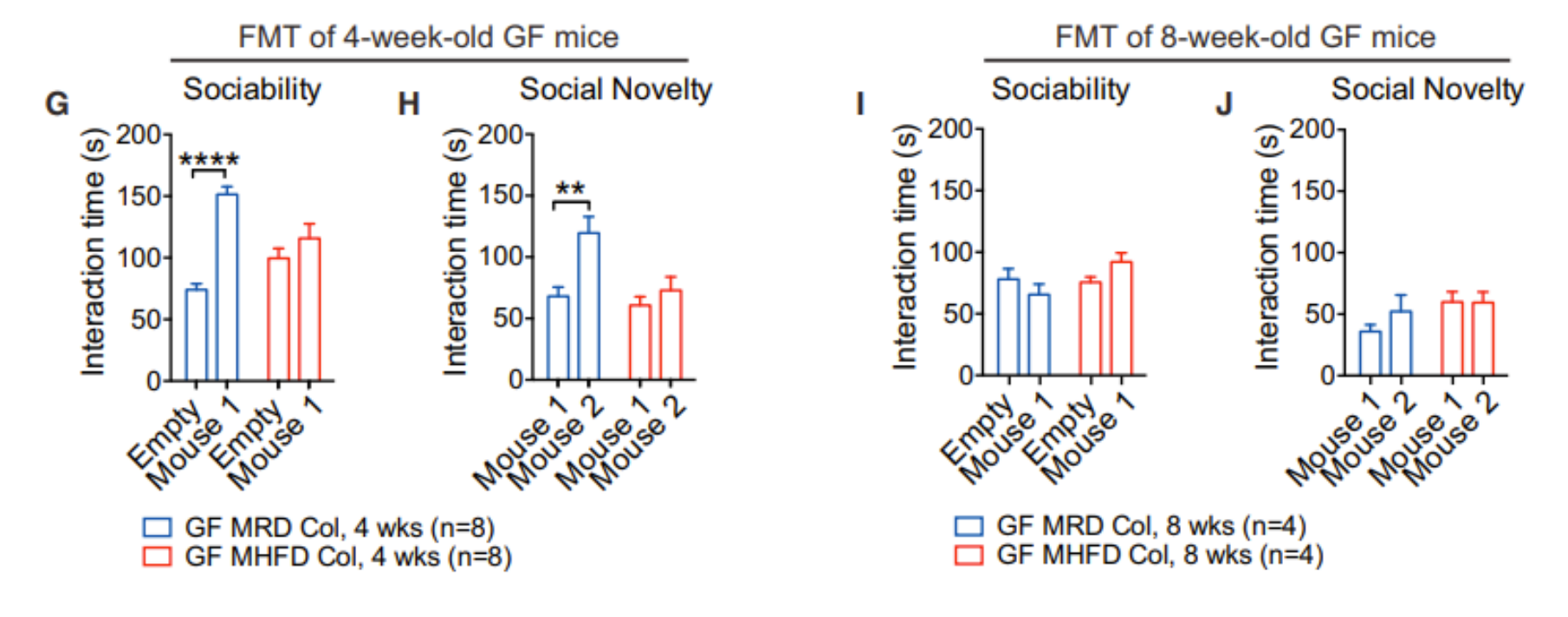
Demonstrates time window where microbiome impacts social behaviour

MHFD induce lack of bacterial diversity in gut microbiome, specifically L. reuteri.

Results

Caitlin Shepherd

Colonisation of germ-free mice



Bacterial Treatment

Lia Lloyd

What we know so far…

- Gut dysbiosis associated with social deficits in MHFD offspring

- Most drastic reduction (>9-fold) visible in Lactobacillus reuteri
  - Metagenomic shotgun sequencing

Bacterial Treatment

Lia Lloyd

Lactobacillus reuteri

- Gram-positive rod, forms chain arrangements
- Does not produce endospores
- Myriad of beneficial effects in humans
- Production antimicrobial molecules (e.g., ethanol)
- Benefits host immune system (e.g., promotes Treg development)
- Importantly, promotes levels of oxytocin
- A hormone with an important role in social behaviour

Seems plausible that decreased L. reuteri is responsible for social defects

- **But how do we prove this?**

Bacterial Treatment

Lia Lloyd

Finding the cause

Addition of different samples to drinking water of mice at weaning age for 4 weeks

1) Heat-killed L. reuteri – no impact
2) Addition L. johnsonii – no impact
3) Live L. reuteri – drastic improvement of sociability and preference for social novelty

Bacterial Treatment

Lia Lloyd

Treatment with *L. reuteri*

- In MHFD offspring, L. reuteri…

- Ameliorates sociability and preference for social novelty
- Rescues stranger interaction-induced long-term synaptic potentiation in the ventral tegmental area of the brain
  - Connections between two neurons become stronger with frequent activation
- Restores reciprocal social interactions
- Increases oxytocin immunoreactivity

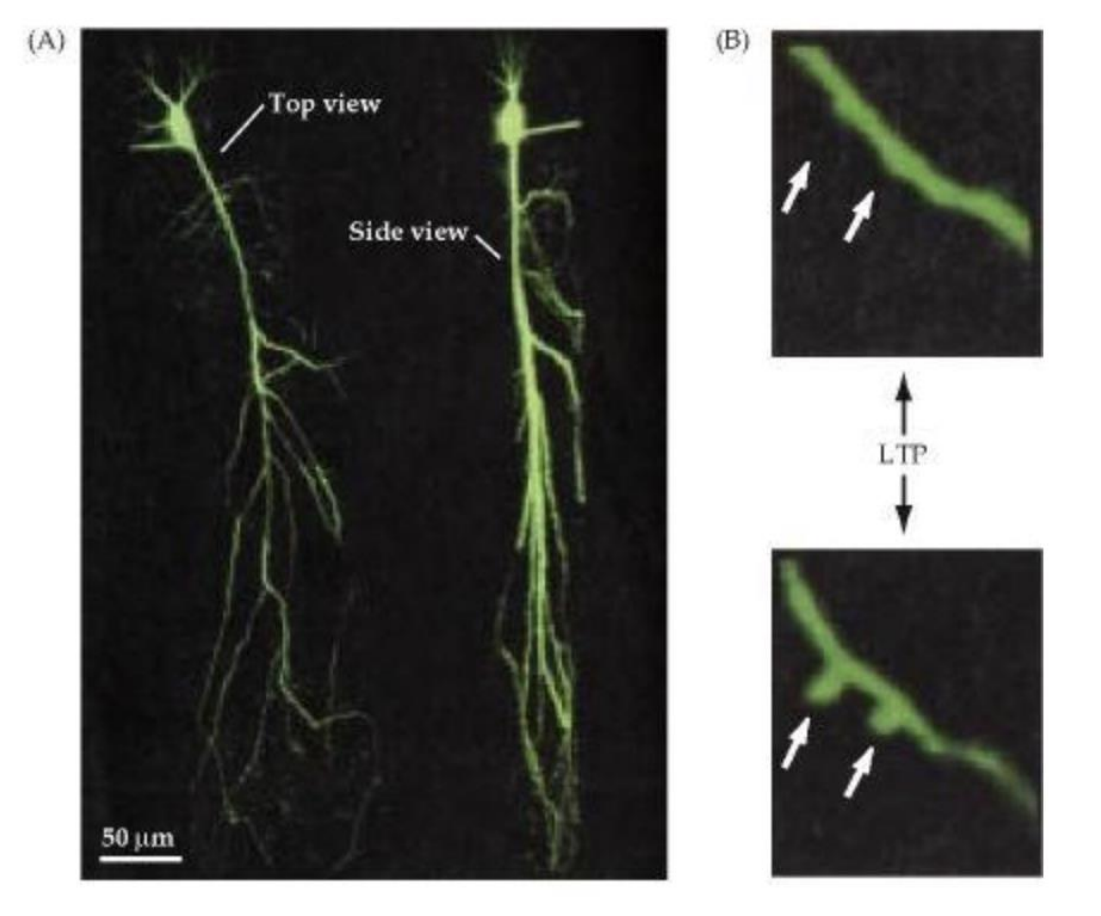
Long-term potentiation

‘The strengthening of synapses from high frequency stimulation results in a lasting increase in signal transmission between neurons’

- Multiple molecular mechanisms:
- Increased probability of glutamate release
- Additional low conductance AMPA receptors
- Replacement of low conductance with high conductance receptors
- Additional high conductance AMPA receptors

Oxytocin

Robin Little



LTP is thought to be a mechanism behind memory and learning.

Oxytocin

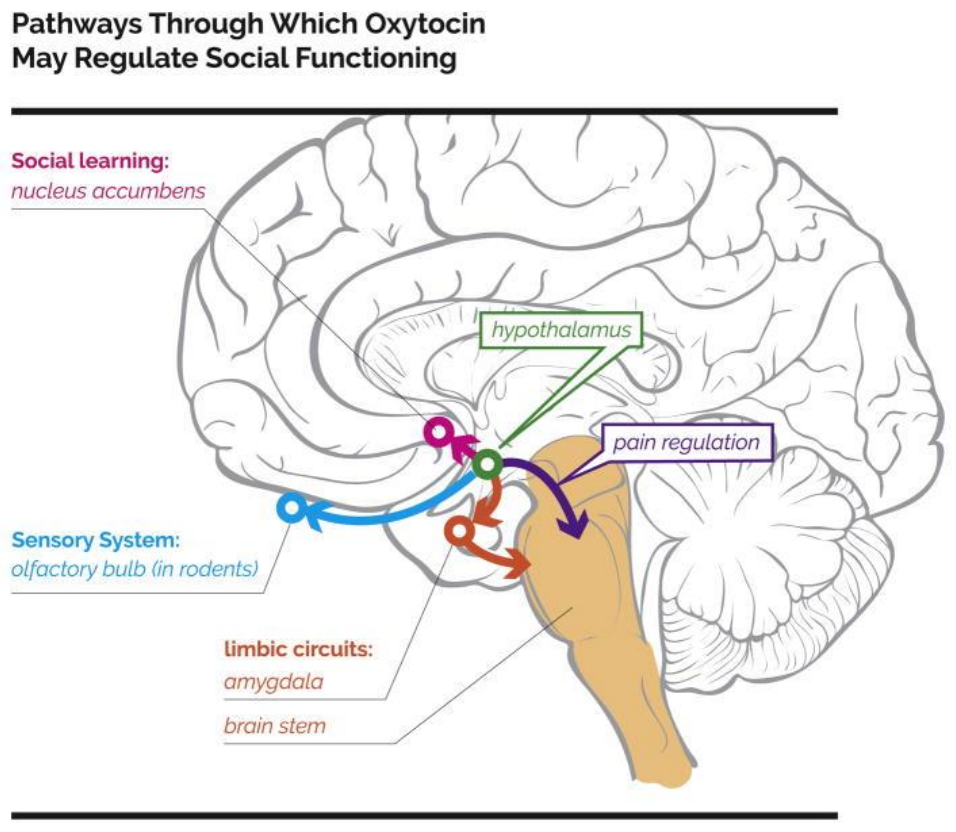
Robin Little

Oxytocin & Social Behaviour

Released in the PVN
Part of a reward system for social behaviour

- Sensory systems
- Limbic circuits
- Social learning

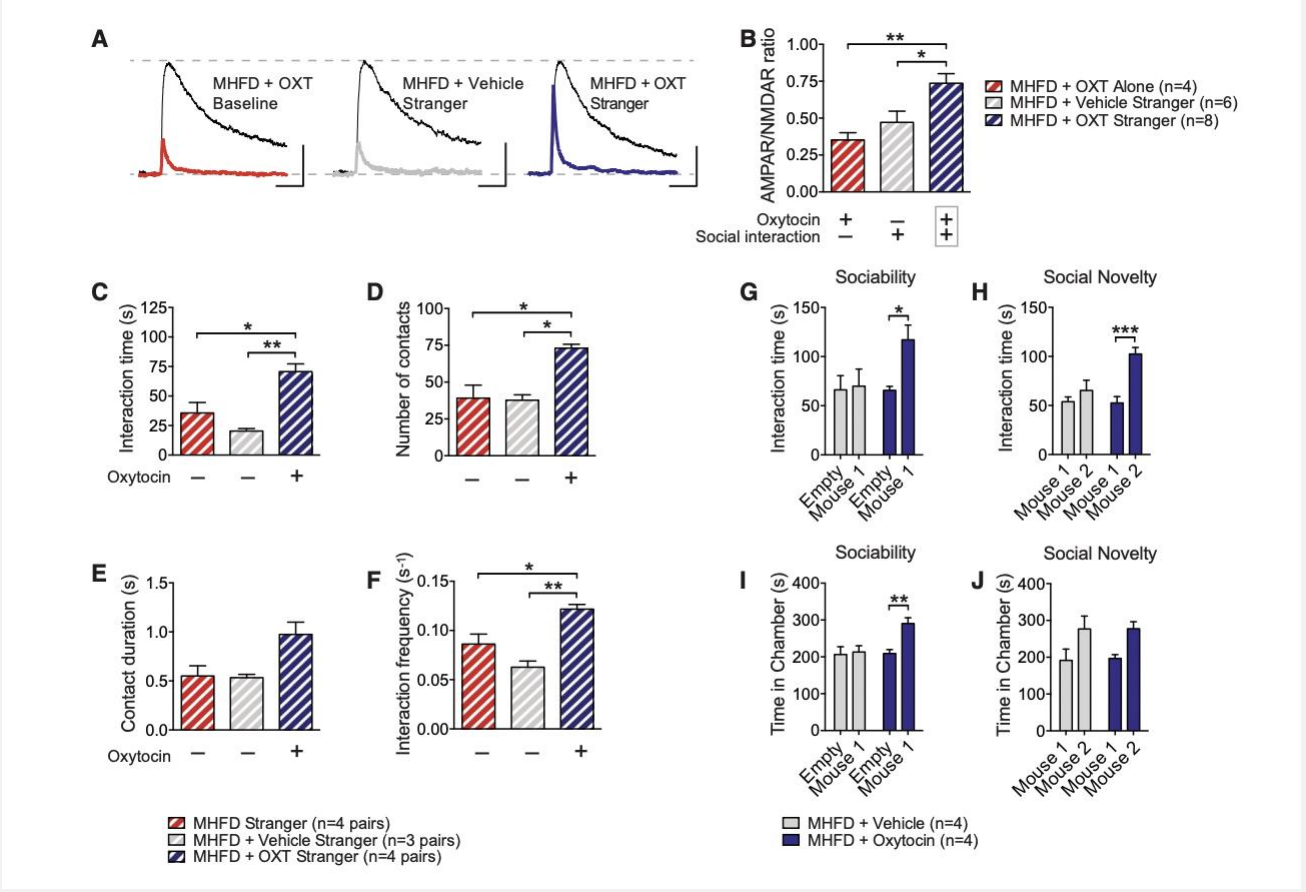
Will administered oxytocin reduce cognitive social defects?



Oxytocin

Robin Little

Treatment



Limitations

Dec Sheftz

Hans Asperger & the origin of autism study

Hans Asperger significantly helped in the conceptualization of autism.

Although his work assisted in discovering the knowledge we currently know today about autism, he was involved in the Nazi regime and encouraged euthanasia amongst children who were seen as ‘genetically unfit’ at Vienna’s Am Spiegelgrund clinic.

Little is known about his life but he was rewarded with career opportunities because of his loyalty to the Nazis, suggesting he was of importance to them.

The Nazis were against the idea of neurodiversity, so people are discussing the validity of the foundational understanding and diagnosis of autism.

Limitations

Dec Sheftz

Science & Society

Genetics plays a major role in determining health, intelligence and characteristics. However, due to socioeconomic factors in certain areas, these phenotypes may be heavily altered.

Both politics and religion/beliefs can directly affect science. Funding and the ability to conduct certain studies may be limited for multiple reasons such as leaders personal bias, cultural issues or religious objections.

Education is another critical factor for science. With little education or the wrong education, scientific understanding and development is massively hindered.

Limitations

Dec Sheftz

Science & Society

Some countries have less strict policies on the use of animals for testing, causing them to go about science in the wrong way and possibly lead to misinformation that could be harmful to humans or other animals.

The relationship between science and society is a lot more prevalent than some may think.

Limitations

Dec Sheftz

Ethical Issues

- People may have issues with mice being used for the testing. In the case of this paper, the maternal mice are being fed a high fat diet to induce social deficits and other issues onto the offspring
- The idea that autism is a ‘disability’ that needs to be treated or prevented
- This paper suggests that a high fat maternal diet has a large impact and directly causes social deficits, possibly making mothers feel guilty even though there are a whole array of factors including genetics that increase the risk of autism
- Altering the gut microbiome led to a restoration of normal social interaction capabilities in some cases. There may be ethical objections to altering gut microbiomes

Limitations

Dec Sheftz

Limitations of the paper

- Only 2 sets of diets were used throughout the experiments. More diets would allow for better analysis on the effect of the maternal high fat diet on the offspring
- It's harder to quantify social interactions between mice than it is compared to humans
- The mice were in a lab, not their natural habitats
- Small numbers of mice were used throughout the experiments making it hard to be a full representation as other factors such as genetics can play a role
- There is a significant contrast in diet and gut microbiome between mice and humans, so although this study can assist in the knowledge of autism causation in humans, it doesn't have any direct implications to whether this also applies to humans

Conclusion

Harsha Shaju

The study highlights the critical role of gut microbiota in shaping brain development and behaviour, and emphasizes the potential of microbial interventions as a novel therapeutic approach for neurodevelopmental disorders. The findings have significant implications for the understanding of the complex relationship between diet, gut microbiota, and brain function.

The study investigates the relationship between maternal diet and gut microbiota and its impact on offspring's behaviour and cognitive abilities. Previous research has shown that maternal diet can affect brain development and behaviour in offspring, but the underlying mechanisms are not well understood. The authors of this study hypothesized that maternal diet-induced changes in gut microbiota can have significant effects on brain development and behaviour in offspring.

Conclusion

Harsha Shaju

- To test this hypothesis, the authors used a mouse model where pregnant female mice were fed a high-fat diet, which has been shown to alter gut microbiota composition. The offspring of these mice were then compared to those of control mice, which were fed a regular diet.
- The researchers observed that the offspring of high-fat diet-fed mice showed deficits in social interaction and cognitive function.
- Next, the researchers performed faecal microbiota transplantation, where they transferred the gut microbiota of control mice into the offspring of high-fat diet-fed mice. The researchers found that this microbial reconstitution reversed the social and synaptic deficits in the offspring of high-fat diet-fed mice.
- These findings suggest that changes in gut microbiota composition due to maternal diet can have a significant impact on brain development and behaviour in offspring and that these effects can be reversed by microbial intervention.

Conclusion

Harsha Shaju

The researchers used a rigorous methodology to investigate their hypothesis. The study had a large sample size, which is crucial to obtain reliable results, and the researchers used both behavioural and molecular techniques to assess the effects of maternal diet and microbial reconstitution on offspring.

The study's findings also have important implications for human health. As maternal diet is a modifiable risk factor, this research suggests that dietary intervention during pregnancy could improve offspring's cognitive function and behaviour.

Moreover, the study highlights the potential of faecal microbiota transplantation as a therapeutic approach for neurodevelopmental disorders, which are becoming increasingly prevalent in our society.

Conclusion

Harsha Shaju

However, the study has some limitations that must be considered. The authors used only one type of high-fat diet, and it is unclear whether other types of high-fat diets would produce similar effects. Additionally, the study did not investigate the long-term effects of microbial reconstitution on offspring, which should be addressed in future research.

Moreover, it is problematic assuming that ASD should be something we ‘prevent’ as most people with ASD would not classify it as a debilitating disease or one that causes any harm.

Conclusion

Harsha Shaju

- In conclusion, the research article by Buffington et al. suggests that maternal diet-induced social and synaptic deficits in offspring can be reversed through microbial reconstitution.
- The study highlights the critical role of gut microbiota in shaping brain development and behavior, and emphasizes the potential of microbial interventions as a novel therapeutic approach for neurodevelopmental disorders. These findings provide important insights into the complex relationship between diet, gut microbiota, and brain function, and may have significant implications for future research in this field.

THANK YOU FOR LISTENING

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