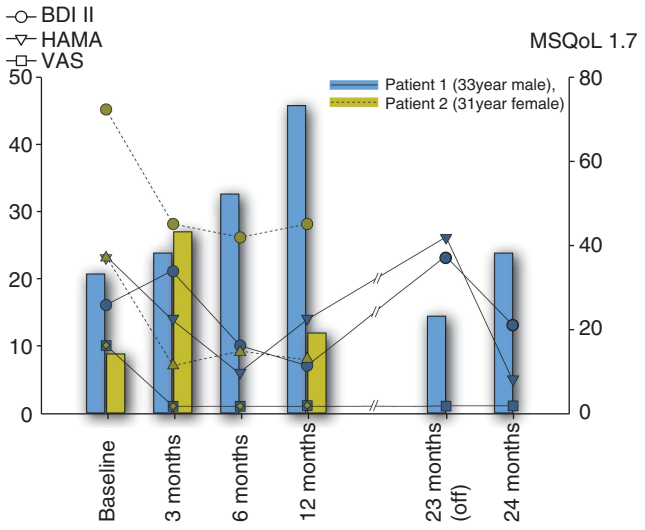

Figure 1. Graphical display of ratings of general quality of life (Modular System for the assessment of Quality of Life subscale 1.7[10]) (bars), mood (Beck Depression Inventory (BDI)), anxiety (Hamilton Anxiety Scale (HAMA)) and craving (VAS) (dashed and solid line) over a 2 and 1 year period of stimulation for patient 1 and 2, respectively. (Shortly before his 2 years follow-up examination, patient 1 reported a significant increase of alcohol and amphetamine consumption, accompanied by an increase of BDI and HAMA values, and an abatement of quality of life. However, an exchange of the battery was followed by a recovery of his psychiatric symptoms. We speculate that unchanged craving might be related to persisting synaptic plasticity[2]). (Funded by DFG (German Research Foundation) KFO 219, Else Kröner-Fresenius-Stiftung and BMBF (German Federal Ministry of Education and Research (01KN1106)).

limbic afferents.[7] Furthermore, experiences of DBS in man also suggest a persisting modulation of synaptic plasticity,[2] as indicated by a lasting abstinence- sustaining effect of NAcc DBS even after explantation of the stimulation device. On the basis of these findings, we hypothesize that NAcc DBS facilitates heroin and methadone abstinence by promoting neuroplastic changes in dopaminergic neurons.

It remains unclear whether the observed beneficial effects allow a generalization to other forms of substance addictions. However, the fact that the patients' comorbid drug consumption did not decline challenges this view. The interpretation of this circumstance in terms of a cross addiction, developing because heroin is no longer available, is also unlikely as the patients did not report increased craving for other psychotropic substances.

From a socio behavioral perspective, the application of DBS could have initially led to a heightened motivation to abstain from drugs. However, the disappointing awareness of persisting private and occupational strains in combination with lacking alternative problem solving strategies might account for the ongoing comorbid drug consume. Patient 2 for example, reported to consume amphetamines mainly to keep her weight in balance. Remaining psychosocial difficulties might likewise explain the renewed decrease of self-perceived quality of life (Modular System for Quality of Life (MSQoL) in patient 2, thereby underlining the importance of psychotherapy accompanying DBS in mental disorders to foster the acquisition of alternative behavioral strategies.[8]

Although the achieved abstinence from heroin in our patients is highly promising, clinical studies with larger samples (as the clinical trial that is to follow) are needed to further support our hypothesis, and to evaluate accumbal DBS as a cost effective treatment option[9] for otherwise treatment resistant drug addiction. Herein, emphasis must be placed on the patients' pattern of comorbid drug consumption.

CONFLICT OF INTEREST

Möller M, Treppmann JF, Bartsch C, Gruendler TOJ, Brosig A, Barnikol UB and Klosterkötter J declare no conflict of interest. Kuhn J has occasionally received honoraria from AstraZeneca, Lilly, Lundbeck and Otsuka Pharma for lecturing at conferences and financial support to travel. Kuhn J received financial support for IIT-DBS studies (not the present investigation) from Medtronic GmbH (Meerbusch, Germany). Lenartz D reports having received financial assistance for travel to congresses from Medtronic AG. Maarouf M has occasionally received honoraria from Medtronic for lecturing at conferences and consulting. Strum V disclosed financial support for studies and travel to congresses, and lecture fees from Medtronic AG and Advanced Neuromodulation Systems INC. He also reported to be a co-holder of patents on desynchronized brain stimulation and shareholder of ANM-GmbH Jülich, a company that intends to develop new stimulators.

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Microbiota is essential for social development in the mouse

Molecular Psychiatry (2014) **19,** 146–148; doi:10.1038/mp.2013.65; published online 21 May 2013

The microbiota–gut–brain axis is an emerging concept in modern medicine informed by the ability of gut microbiota to alter brain and behaviour.[1] Although some clinical studies have revealed altered gut microbiota composition in patients with neurodevelopmental disorders such as autism,[2,3] the specific

contributions of microbiota in early life to the development and programming of the various facets of social behaviour has not been investigated.

Germ-free (GF) mice have been critical in assessing the role of microbiota in all aspects of physiology. Indeed, recent studies in GF mice report increases in neuroendocrine responses to stress,[4,5] altered neurotrophin levels in the hippocampus and amygdala,[5–7] reduced anxiety[5–7] and non-spatial memory,[8] and altered monoamine neurotransmitter levels in the brain.[5,6] Interestingly, many of the deficits are specific to males[4] in which there are higher incidence rates of neurodevelopmental disorders relative to females. Here, we examined the effects of GF rearing conditions through early life and adolescence on social behaviour in adulthood.

Mice, like humans, are a social species and have a natural propensity to seek out the security and pleasure afforded by stable social scenarios. Social motivation and preference for social novelty in mice can be assessed in the three-chambered sociability test.[9] Our initial findings in this test revealed significant social impairments in GF mice, particularly in males, as indicated by a lack of the normal preference for time spent in a chamber containing a mouse versus the alternative empty chamber (GF × chamber interaction: $F(1,57) = 5.35$, $P < 0.05$; Supplementary Figures 1a–c). This was accompanied by reduced preference for novel social situations, where GF mice did not demonstrate the normal increase in time spent investigating a novel over a familiar mouse, which resembles social cognition deficits observed in patients with neurodevelopmental disorders (GF × chamber interaction: $F(1,57) = 5.86$, $P < 0.01$; Supplementary Figures 1d–f).

To substantiate these results and to assess the capacity for post-weaning bacterial colonisation of the GF gut (GFC) to reverse the observed social deficits, we repeated the test in a different male cohort. As expected, GF mice exhibited robust deficits including social avoidance (GF × chamber interaction: $F(1,20) = 12.41$, $P < 0.001$; Figures 1a–c), and diminished preference for social novelty relative to conventionally colonised (CC) mice (GF × chamber interaction: $F(1,20) = 4.45$, $P < 0.05$; Figures 1d–f). These effects were not influenced by changes in general locomotor activity, as any decrease in chamber entries was specific to the social chamber (Supplementary Figure 2). Intriguingly, whereas GFC reversed the observed social avoidance, it had no effect on social cognition impairments. This indicates that although the effects of GF rearing on the latter behaviour are permanently established in the pre-weaning period, the development of social avoidance in GF mice is more amenable to microbial-based interventions in later life.

In addition to symptoms of reduced social motivation, children with autism exhibit poor social and communication skills and repetitive behaviours. To establish whether the degree of information transfer during social interaction is disrupted in GF mice, performance in the social transmission of food preference test was assessed. GF mice spent a decreased proportion of time engaged in social investigation ($F(2,20) = 7.51$, $P < 0.005$; Figure 1g) and substantially greater proportion of time engaged in repetitive self-grooming behaviour ($F(2,20) = 11.91$, $P < 0.001$; Figure 1h) during social interaction. These behaviours were normalised following GF bacterial colonisation, confirming the involvement of microbiota in modulation of these behaviours. However, despite the reduction in social investigation times, the quality of information transfer during the interaction was not affected in GF mice, as they displayed normal preference for the novel food (food to which cage-mate was exposed prior to social interaction) in the subsequent food choice test conducted immediately after the social interaction and 24 h later (Figures 1i and j), indicating that the ability to process information *per se* during social interaction is not affected in GF mice.

This study shows for, what is to our knowledge, the first time that microbiota are crucial for the programming and presentation of distinct normal social behaviours, including social motivation and preference for social novelty, while also being important regulators of repetitive behaviours. Given that these facets of behaviour are impaired in neurodevelopmental disorders such as schizophrenia and autism[10] and with a similar male preponderance, these data may have implications for our understanding of the genesis of neurodevelopmental disorders of altered sociability. A better understanding of the mechanisms underlying these social deficits, which may include modulation of immune cell cytokines release, changes in vagal nerve activity and neuroendocrine function, could potentially lead to the emergence of novel and more effective therapies to combat symptoms in the social domain.

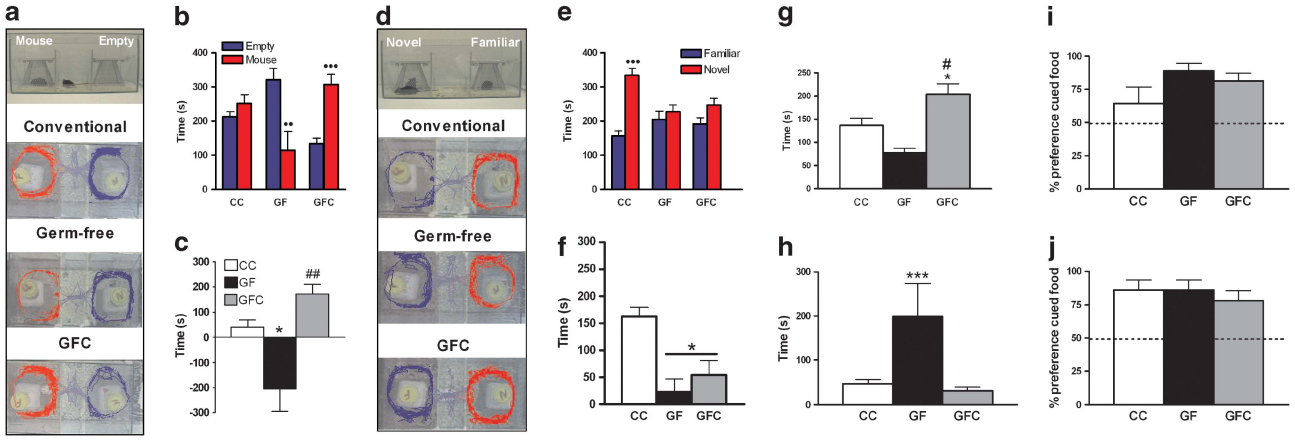

Figure 1. Effects of germ-free (GF) rearing and germ-free bacterial colonisation (GFC) on social behaviours in male mice. In the three-chambered sociability test, GF mice failed to show the normal preference for the social chamber displayed by conventionally colonised (CC) and GFC groups during trial 2, as seen in the automated tracking images (**a**), the time spent in each chamber (**b**) and the difference between time spent in mouse and empty chambers (**c**). This social avoidance was reversed in GFC mice (**b** and **c**). GF and GFC mice also failed to show normal preference for social novelty displayed by CC mice during trial 3, as seen in the automated tracking images (**d**), the time spent in each chamber (**e**) and the difference between time spent in the chambers containing a novel and familiar mouse (**f**). In the social transmission of food preference test, GF rearing conditions altered social investigation time (**g**) and grooming time (**h**) during social interaction with demonstrator mice. There was no effect on the preference for cued food immediately after social interaction (**i**) and 24 h later (**j**). $^{••}P < 0.01$, $^{•••}P < 0.001$ versus opposite chamber; repeated-measures analysis of variance followed by *post-hoc* Newman–Keuls test ($n = 7–13$); $^{*}P < 0.05$, $^{***}P < 0.001$ versus CC; $^{\#}P < 0.01$, $^{\#\#}P < 0.001$ versus GF; one-way analysis of variance followed by *post-hoc* Newman–Keuls test ($n = 5–13$).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Dr Gerard O'Keeffe for helpful comments on the paper. We also thank Pat Fitzgerald and Frances O'Brien for their technical contributions to the study. The Alimentary Pharmabiotic Centre is a research centre funded by Science Foundation Ireland (SFI), through the Irish Government's National Development Plan. The authors and their work were supported by SFI (grant nos. 02/CE/B124 and 07/CE/B1368).

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

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CSF biomarkers for Alzheimer's pathology and the effect size of *APOE* ε4

Molecular Psychiatry (2014) **19,** 148–149; doi:10.1038/mp.2013.18; published online 19 February 2013

New research and clinical criteria for Alzheimer's disease (AD) have recently been proposed, which include biomarker information on Alzheimer's plaque and tangle pathology, or AD-typical structural brain changes, as supporting or essential elements of an AD diagnosis.[1–3] In a large group of patients with both genetic and cerebrospinal fluid (CSF) biomarker data, we here show that biomarker-assisted diagnosis-making almost doubles the effect size of the association between the ε4 variant of the apolipoprotein E (*APOE*) gene and AD.

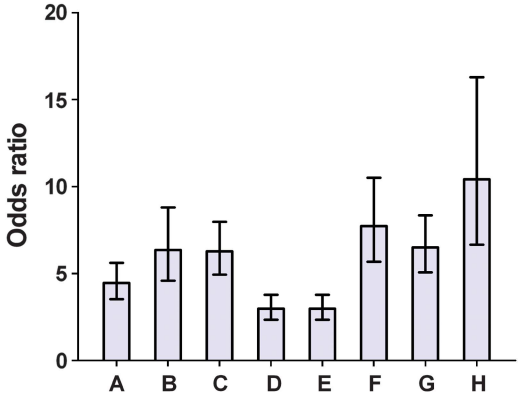

Figure 1. Odds ratios for a positive *APOE* ε4 carrier status based on (A) clinical diagnosis, comparing patients with clinical AD with dementia at inclusion or follow-up ($n=596$) versus all other diagnostic groups ($n=749$), (B) clinical diagnosis, comparing patients with clinical AD with dementia at inclusion or follow-up ($n=596$) with cognitively normal subjects ($n=251$), (C) CSF Aβ42, comparing subjects with CSF Aβ42 below ($n=779$) and above ($n=563$) 546 ng/l, (D) CSF T-tau, comparing subjects with CSF T-tau above ($n=676$) and below ($n=662$) 446 ng/l, (E) CSF P-tau, comparing subjects with CSF P-tau above ($n=497$) and below ($n=759$) 79 ng/l, (F) CSF P-tau/Aβ42 ratio, comparing subjects with CSF P-tau/Aβ42 above and below 0.15, (G) CSF biomarker signatures, comparing subjects with an AD-indicative CSF signature with regards to all three biomarkers T-tau, P-tau and Aβ42, and subjects with a normal complete profile (cut-points specified above) and (H) CSF biomarker signatures in addition to clinical diagnosis, comparing patients with clinical AD and an AD-indicative CSF biomarker signature versus cognitively normal subjects with normal CSF biomarker results (cut-points specified above). Note that columns C-G are derived without any clinical information.

We included clinically diagnosed patients with either AD dementia ($n=309$) or mild cognitive impairment (MCI) due to AD ($n=287$), cognitively normal controls ($n=251$) and patients with MCI who remained stable over at least 2 years ($n=399$) or developed dementias other than AD ($n=99$) (Table 1, Supplementary Material). All had *APOE* ε2/ε3/ε4 genotypes and results on the CSF biomarkers total tau (T-tau), phosphorylated tau (*P*-tau) and the 42-amino-acid isoform of amyloid-β (Aβ42) determined. These CSF biomarkers reflect the core elements of Alzheimer's pathology[4] and are strongly associated with AD in cross-sectional as well as longitudinal follow-up studies (Supplementary Material).[5,6]

AD dementia and MCI-AD patients were first pooled into one clinical AD group ($n=596$) and compared with all remaining categories that were designated non-AD ($n=749$). A positive *APOE* ε4 carrier status (one or two ε4 alleles) was overrepresented in the AD group and yielded an odds ratio (OR) of 4.45 (95% confidence interval (CI) 3.52–5.62) for a clinical diagnosis of AD at inclusion or follow-up (Figure 1). This OR is similar to the AlzGene meta-analysis of *APOE* (3.68, 95% CI 3.30–4.11, www.alzgene.org/meta.asp?geneID=83, November 2012 freeze). Similarly, we tested the association of *APOE* ε4 with AD, comparing the 596 AD patients with the 251 cognitively normal controls, which resulted in an OR of 6.35 (95% CI 4.59–8.80).

Disregarding the clinical diagnoses and subgrouping all subjects into amyloid-positive, defined as CSF Aβ42 $<546\,\mathrm{ng\,l^{-1}}$ ($n=779$), and amyloid-negative, defined as CSF Aβ42 $\geqslant 546\,\mathrm{ng\,l^{-1}}$ ($n=563$) (see Supplementary Material for details on cut-point determination), gave an OR for *APOE* ε4 as high as