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Post-weaning social isolation of rats leads to long-term disruption of the gut microbiota-immune-brain axis

Fionn Dunphy Doherty [a,1], Siobhain M. O'Mahony [b,c,1], Veronica L. Peterson [c], Orla O'Sullivan [c,d], Fiona Crispie [d], Paul D. Cotter [c,d], Peter Wigmore [a], Madeleine V. King [a], John F. Cryan [b,c,2], Kevin C.F. Fone [a,2,*]

[a] School of Life Sciences, Medical School, Queen's Medical Centre, The University of Nottingham, Nottingham NG7 2UH, United Kingdom
[b] Department of Anatomy and Neuroscience, University College Cork, Ireland
[c] APC Microbiome Institute, University College Cork, Ireland
[d] Teagasc Food Research Centre, Moorepark, Fermoy, Ireland

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ABSTRACT

Early-life stress is an established risk for the development of psychiatric disorders. Post-weaning isolation rearing of rats produces lasting developmental changes in behavior and brain function that may have translational pathophysiological relevance to alterations seen in schizophrenia, but the underlying mechanisms are unclear. Accumulating evidence supports the premise that gut microbiota influence brain development and function by affecting inflammatory mediators, the hypothalamic–pituitaryadrenal axis and neurotransmission, but there is little knowledge of whether the microbiota-gut-brain axis might contribute to the development of schizophrenia-related behaviors. To this end the effects of social isolation (SI; a well-validated animal model for schizophrenia)-induced changes in rat behavior were correlated with alterations in gut microbiota, hippocampal neurogenesis and brain cytokine levels. Twenty-four male Lister hooded rats were housed in social groups (group-housed, GH, 3 littermates per cage) or alone (SI) from weaning (post-natal day 24) for four weeks before recording open field exploration, locomotor activity/novel object discrimination (NOD), elevated plus maze, conditioned freezing response (CFR) and restraint stress at one week intervals. Post-mortem caecal microbiota composition, cortical and hippocampal cytokines and neurogenesis were correlated to indices of behavioral changes. SI rats were hyperactive in the open field and locomotor activity chambers traveling further than GH controls in the less aversive peripheral zone. While SI rats showed few alterations in plus maze or NOD they froze for significantly less time than GH following conditioning in the CFR paradigm, consistent with impaired associative learning and memory. SI rats had significantly fewer BrdU/NeuN positive cells in the dentate gyrus than GH controls. SI rats had altered microbiota composition with increases in Actinobacteria and decreases in the class Clostridia compared to GH controls. Differences were also noted at genus level. Positive correlations were seen between microbiota, hippocampal IL-6 and IL-10, conditioned freezing and open field exploration. Adverse early-life stress resulting from continuous SI increased several indices of 'anxiety-like' behavior and impaired associative learning and memory accompanied by changes to gut microbiota, reduced hippocampal IL-6, IL-10 and neurogenesis. This study suggests that early-life stress may produce long-lasting changes in gut microbiota contributing to development of abnormal neuronal and endocrine function and behavior which could play a pivotal role in the aetiology of psychiatric illness.

1. Introduction

Exposure to early-life stress is an established risk factor for the development of several common psychiatric conditions; including anxiety-related disorders, schizophrenia, post-traumatic stress disorder and depression (Heim et al., 1997). Furthermore, stress is the strongest predictor of developing depression in clinical

Abbreviations: ANOVA, analysis of variance; BrdU, 5-bromo-2′-deoxyuridine; CFR, conditioned freezing response; GH, Group-housed; HPA, hypothalamic pituitary-adrenal axis; NeuN, neuronal nuclei; NOD, novel object discrimination; PFC, prefrontal cortex; SI, social isolation; SEM, standard error of the mean.

* Corresponding author.
E-mail address: kevin.fone@nottingham.ac.uk (K.C.F. Fone).

[1] Joint First authors.
[2] Joint Senior Authors.

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populations (Kendler et al., 1993), and developmental rodent models of psychiatric illness often contain a stress component to attempt to provide translational relevance (Nestler and Hyman, 2010). In rodents adverse early-life events modify brain development, affecting resultant adult behavior and often producing neurobiological sequelae that resemble changes seen in depression and schizophrenia which may therefore contribute to development of these common psychiatric disorders (Lapiz et al., 2003; Pryce et al., 2005).

Social isolation of rat pups from weaning (hereafter referred to as SI) is an established neurodevelopmental model producing robust behavioral, neurochemical and anatomical changes reminiscent of several core features of schizophrenia and depression (Fone and Porkess, 2008; Jones et al., 2011). These include attenuated exploratory habituation to a novel arena (Gentsch et al., 1982; Meffre et al., 2012; Sahakian et al., 1977), enhanced 'anxiety-related' behavior in the elevated plus maze (Parker and Morinan, 1986; Weiss et al., 2004) and reduced social interaction (McIntosh et al., 2013; Watson et al., 2016; Wongwitdecha and Marsden, 1996). SI rats also show impaired learning and memory in multiple cognitive domains including; visual learning and memory (novel object discrimination) pre-attention processing (prepulse inhibition of acoustic startle) and associative learning (conditioned freezing response, CFR) (Bakshi et al., 1998; Bianchi et al., 2006; Fone and Porkess, 2008; Gaskin et al., 2016; McIntosh et al., 2013; Meffre et al., 2012). SI also modifies the hypothalamic pituitary-adrenal (HPA) axis stress response. However basal levels of adrenocorticotrophic hormone and corticosterone have been reported to be increased (Weiss et al., 2004) or unaltered (Scaccianoce et al., 2006; Schrijver et al., 2002), which might depend on the strain of rat (Malkesman et al., 2006) or the severity of the isolation procedure (e.g. use of sawdust bedding or housing on a grid floor) (Fone and Porkess, 2008). One group (Serra et al., 2005) reported SI caused hypersensitivity of the pituitary gland to intraventricular injection of corticotrophin-releasing hormone and impaired glucocorticoid receptor mediated negative feedback of the HPA axis, analogous to suppression of feedback inhibition seen in severe depression. Furthermore, Muchimapura et al. (2002) demonstrated that SI can affect the stress response by altering neurodevelopment of the serotonergic innervation of the hippocampus.

Despite a wealth of research, the mechanisms underlying the developmental changes produced by SI are not established. One possible contributory factor, which has so far received little investigation, is that isolation rearing modifies the microbiota-gut-brain axis thereby disrupting normal neural, endocrine, metabolic and immunological bicommunication between gut microbiome and the brain (Jones et al., 2006). This could then adversely affect neuronal development and adult behavior (see reviews, (Cryan and Dinan, 2012; Parashar and Udayabanu, 2016; Sarkar et al., 2016)). Indeed recent research has implicated alterations in the composition of gut microbiota as a potential factor in the aetiology of a number of central nervous system disorders; including autism (Williams et al., 2011), anxiety and depression (Foster and McVey Neufeld, 2013; Naseribafrouei et al., 2014) but there has been a paucity of research on any link between gut microbiota and schizophrenia (Dinan et al., 2014) despite association with autoimmunity and gastrointestinal disorders (Severance et al., 2016). Early studies of microbiota established that germ-free mice show increased stress reactivity (Sudo et al., 2004), repetitive self-grooming and reduced social interaction which are all reversed by colonization of the gut (Desbonnet et al., 2014). Conversely studies also demonstrate that exposure of rodents to a stressor alters microbiota resulting in an associated increase in pro-inflammatory cytokines (Bailey et al., 2011; Galley and Bailey, 2014).

For example, maternal separation of rat pups not only enhances anxiety-related behavior and cytokine levels but also changes gut microbiota (O'Mahony et al., 2009) and treatment with probiotics has a comparative effect to the SSRI citalopram; attenuating some of these changes (Desbonnet et al., 2010). However, there is limited data on the role of the microbiota in any animal model for schizophrenia. To this end we characterised the impact of housing rats in SI from weaning on a behavioral tasks (selected for translational relevance to core symptoms seen in schizophrenia) evaluating performance in cognitive, 'anxiety-like' and stress paradigms and correlated these with changes in gut microbiota, hippocampal and prefrontal cortical (PFC) cytokine, plasma corticosterone levels and hippocampal neurogenesis (BrdU/NeuN expressing neurones). Associations are made between changes in specific behavioral phenotypes and gut microbiota in rat littermates either housed in small social groups (GH) or in SI and the potential translational relevance of these findings to common neurodevelopmental psychiatric disorders is discussed.

2. Methods

2.1. Animals

Twenty-four male Lister hooded rats (from four equal sized litters) were obtained from Charles River UK on post-natal day 24 and weaned on arrival. Each litter was divided such that half the pups were group-housed (GH) and the other half reared alone (SI). Rats were housed in plastic cages (32×51 cm, GH or 25×42, SI) containing sawdust bedding with metal grid lids, and had visual, auditory and olfactory contact with conspecifics in the same holding room. Rats were weighed once weekly (when bedding was changed). Food and water were available *ad libitum* and rats were kept on a twelve hour light/dark cycle (lights on 07.00 h) at constant temperature (21 ± 2 °C) and humidity (45 ± 1 5%). On post-natal day 56 rats received 5-bromo-2′-deoxyuridine (BrdU, a marker of cell proliferation, Sigma Aldrich, 150 mg/kg, i.p.) and housed for a further week before behavioral testing began (Fig. 1). All behavioral studies were conducted between 08.00 and 17.00 h with SI and GH rats being analysed in a random order to minimise any temporal effect, and before each test the arena was cleaned with 20% ethanol and dried to remove odour cues. All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act (1986), with approval of the University of Nottingham Local Ethical Review Committee, and conformed to the ARRIVE guidelines (Kilkenny et al., 2010; McGrath et al., 2010). Behavioral studies were conducted during the light phase of the circadian cycle to enable comparison with all our previous work with this model and the majority of other publications. Although there may be sex differences in behavioral and neuroendocrine responses following SI, this was not the primary focus of the current studies which were therefore performed on male rats to minimise the number of animal required according to the 3R's principle.

2.2. Open field behavior

As an ethological index of anxiety, behavior was recorded in a novel circular open field (on post-natal day 59, Fig. 1.). Rats were placed in the centre of the unfamiliar arena (75 cm diameter with 45 cm high walls, lined with grey card) at 120Lux for 15 min and behavior recorded using computerised tracking (Noldus, Ethovision XT ver7.1). Ambulatory speed and distance travelled in the peripheral and central zone (15 cm from the wall defined on Ethovision) together with time spent rearing and body grooming and the number of faecal boli were measured as indices of 'anxiety levels'.

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