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Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats

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Abstract
Background and aims: Establishment of the gut microbiota is one of the most important events in early life and emerging evidence indicates that the gut microbiota influences several aspects of brain functioning, including reactivity to stress. To better understand how the gut microbiota contributes to a vulnerability to the stress-related psychiatric disorders, we investigated the relationship between the gut microbiota, anxiety-like behavior and HPA axis activity in stress-sensitive rodents. We also analyzed the monoamine neurotransmitters in the brain upper structures involved in the regulation of stress and anxiety.
Methods: Germfree (GF) and specific pathogen free (SPF) F344 male rats were first subjected to neurological tests to rule out sensorimotor impairments as confounding factors. Then, we

Abbreviations: ACTH, adrenocorticotropic hormone; CORT, corticosterone; CRF, corticotropin releasing factor; DA, dopamine; DG, dentate gyrus; DOPAC, dihydroxyphenyl acetic acid; DTT, dithiothreitol; GF, germfree; GR, glucocorticoid receptor; 5-HIAA, 5-hydroxyindole acetic acid; HPA, hypothalamic pituitary adrenal; HPLC, high performance liquid chromatography; 5-HT, 5-hydroxytryptamine (serotonine); HVA, homovanillic acid; NE, norepinephrine; OF, open-field; PBS, phosphate buffered saline; PVN, paraventricular nucleus; SSC, saline sodium citrate; SPF, specific pathogen free.

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examined the behavior responses of rats to social interaction and open-field tests. Serum corticosterone concentrations, CRF mRNA expression levels in the hypothalamus, glucocorticoid receptor (GR) mRNA expression levels in the hippocampus, and monoamine concentrations in the frontal cortex, hippocampus and striatum were compared in rats that were either exposed to the open-field stress or not.

Results: GF rats spent less time sniffing an unknown partner than SPF rats in the social interaction test, and displayed a lower number of visits to the aversive central area, and an increase in latency time, time spent in the corners and number of defecations in the open-field test. In response to the open-field stress, serum corticosterone concentrations were 2.8-fold higher in GF than in SPF rats. Compared to that of SPF rats, GF rats showed elevated CRF mRNA expression in the hypothalamus and reduced GR mRNA expression in the hippocampus. GF rats also had a lower dopaminergic turnover rate in the frontal cortex, hippocampus and striatum than SPF rats.

Conclusions: In stress-sensitive F344 rats, absence of the gut microbiota exacerbates the neuroendocrine and behavioral responses to acute stress and the results coexist with alterations of the dopaminergic turnover rate in brain upper structures that are known to regulate reactivity to stress and anxiety-like behavior.

1. Introduction

In mammals, the underlying mechanisms of stress, fear and anxiety are shaped in early life both by genetic and environmental factors, and disturbances of brain development and maturation could contribute to a later vulnerability to psychiatric disorders such as hyper-responsiveness to stress, depression and addiction (Vazquez et al., 2005; Heim et al., 2008; Enoch et al., 2010). Also in early life, mammals establish their gut microbiota, and this process is highly influenced by environmental factors. In normal conditions, these gut symbionts, whose collective genomes encode a vast array of diverse functional genes (Lozupone et al., 2012), affect various aspects of host physiology, including brain development and functions (Grenham et al., 2011; Forsythe and Kunze, 2013). In supporting the role of intestinal bacteria in gut—brain connections, it was shown recently in a cohort of healthy adults that chronic consumption of a fermented milk product with probiotic, known to alter gut microbiota metabolism, modulated the activity of several brain areas involved in sensory perception and emotion (Tillisch et al., 2013). Emerging evidence also indicates the connections between the gut microbiota and neurodevelopmental disorders. Gut microbiota composition and metabolism of autistic children are different from those of the general population (Yap et al., 2010; Finegold et al., 2012), and children with regressive-onset autism reported improvement of gastrointestinal disturbances and behavior features following a treatment with a non-absorbable oral antibiotic aimed at modulating the gut microbiota (Sandler et al., 2000).

Recently, animal experiments showed that germfree (*i.e.* devoid of gut microbiota, GF) adult male BALB/c mice had an elevated basal level of CRF gene expression in the hypothalamus and over-reacted to an acute restraint stress by a hyper-secretion of adrenocorticotropic hormone (ACTH) and corticosterone (CORT), compared with specific pathogen-free (SPF) counterparts (Sudo et al., 2004). Such a maladaptive response in GF mice to stress was partly corrected by gut microbiota reconstitution with fecal bacteria of the SPF mice. Interestingly, this treatment was only effective when the GF mice were colonized at six but not at 14 weeks of age, suggesting the existence of a critical period for programming the reactivity of the hypothalamic—pituitary—adrenal (HPA) axis (Sudo et al., 2004). In another experiment, the same authors showed that GF BALB/c mice expressed a higher anxiety-like behavior than SPF counterparts in the open-field (OF) and marble-burying tests, and had lower turnover rates of monoamines in several brain regions (Nishino et al., 2013). In contrast, studies conducted with other mice models, namely Swiss adult males and females and NMRI adult males, showed that a GF status was associated with reduced anxiety-like behavior in the elevated plus maze and light—dark choice tests, and increased the striatal and hippocampal turnover of monoamines (Neufeld et al., 2011; Diaz-Heijtz et al., 2011; Clarke et al., 2013). In some of those studies, an HPA axis hyper-activity in basal conditions (Neufeld et al., 2011) or in response to stress (Clarke et al., 2013) was described. The joint presence of HPA axis hyper-activity and anxiolysis is unexpected because stress is commonly associated with anxiety (Shekhar et al., 2005; Laryea et al., 2012). Therefore, the aim of the present study was to clarify the impact of the GF status on HPA axis reactivity to stress and on anxiety by testing the neuroendocrine and behavioral responses of animals to novel challenges. Specifically, we subjected GF and SPF adult male F344 rats to a social interaction experience and to an OF test, and we measured (i) the serum CORT concentration, (ii) the expression level of the CRF gene in the hypothalamus and of the glucocorticoid receptor (GR) gene in the hippocampus, and (iii) monoamine concentrations in the brain upper structures involved in the regulation of stress and anxiety (frontal cortex, hippocampus and striatum). In addition, we carried out a neurological examination to ascertain that behavioral differences between GF and SPF rats could not result from sensorimotor development impairments. We opted for the rat as an animal model to obtain additional data in another species than the mouse, which was exclusively used until now, and because of its more complex behavior (Baker, 2011). The strain F344 was chosen as a hyper-responsive strain to stress (Sarrieau et al., 1998) and we focused the study on the male sex to avoid interferences between estrous