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Research report

# Autistic-like behavioural and neurochemical changes in a mouse model of food allergy

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## HIGHLIGHTS

- Food allergy in mice reduced social behaviour, increased repetitive behaviour and impaired spatial memory.
- Abnormalities of the serotonergic system in the intestines occurred.
- c-Fos expression increased in obitofrontal cortex and decreased hypothalamic paraventricular nucleus.
- Dopaminergic system was dampened in prefrontal cortex and enhanced in amygdala.

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## ABSTRACT

Food allergy has been suggested to contribute to the expression of psychological and psychiatric traits, including disturbed social behaviour and repetitive behaviour inherent in autism spectrum disorders (ASD). Most research in this field receives little attention, since fundamental evidence showing direct effects of food allergic immune responses on social behaviour is very limited. In the present study, we show that a food allergic reaction to cow's milk protein, induced shortly after weaning, reduced social behaviour and increased repetitive behaviour in mice. This food allergic reaction increased levels of serotonin (5-hydroxytryptamine; 5-HT) and the number of 5-HT positive cells, and decreased levels of 5-hydroxyindoleacetic acid (5-HIAA) in the intestine. Behavioural changes in food allergic mice were accompanied by reduced dopaminergic activity in the prefrontal cortex. Furthermore, neuronal activation (c-Fos expression) was increased in the prefrontal cortex and reduced in the paraventricular nucleus of the hypothalamus after exposure to a social target. We hypothesize that an intestinal allergic response regulates complex, but critical, neuroimmune interactions, thereby affecting brain circuits involved in social interaction, repetitive behaviour and cognition. Together with a genetic predisposition and multiple environmental factors, these effects of allergic immune activation may exacerbate behavioural abnormalities in patients with ASD.



## 1. Introduction

The intestinal tract continuously encounters foreign antigens and is therefore the most complex organ of the immune system. The majority of these antigens are harmless food antigens to which the body has formed a tolerogenic reaction. Genetic predisposition and environmental factors, however, can abrogate tolerance towards food allergens, leading to a Th2-directed immune response characterized by production of allergen-specific immunoglobulins during sensitization, and mast cell degranulation upon a second exposure to the allergen [1].

The intestinal tract is not only distinguished by its crucial immune function, but also exerts an important neurological

*Abbreviations:* 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; ASD, autism spectrum disorders; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EC, enterochromaffin cell; DSS, dextran sodium sulphate; HVA, homovanillic acid; Ig, immunoglobulin; mMCP-1, mouse mast cell protease-1; oPFC, orbital prefrontal cortex; PVN, hypothalamic paraventricular nucleus; SERT, serotonin transporter; TRP, L-tryptophan.

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function and is called 'the second brain' because of its abundant amount of enteric nerves. Evidence is emerging that intestinal immune disturbances can signal to the brain through various pathways, affecting behaviour and emotion [2]. Food allergy has been suggested to be one of the intestinal triggers that can contribute to the expression of various psychological and psychiatric traits, including anxiety, depression, migraine, schizophrenia, attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) [3–6]. Supporting the hypothesis that food allergy can affect mental disorders of psychosocial relevance, Meldrum et al. [7] recently observed social neurodevelopmental abnormalities in food allergic children at 18 months of age. Diagnosis of food allergy was associated with enhanced internalizing behaviour and a trend towards low social emotional scores. Intestinal problems are often reported in children with ASD [8,9] and milk intake was found to be a predictor of constipation [10]. Furthermore, a (gluten and) milk protein free diet is suggested to improve autistic behaviours [11–13] and to restore the increased intestinal permeability observed in these children [14]. Preclinical studies on neurological effects of food allergy are limited. Mice immunized to ovalbumin (OVA) displayed increased anxiety 1 h after oral challenge with OVA [15]. Moreover, c-Fos staining of the paraventricular nucleus (PVN) of the hypothalamus and central nucleus of the amygdala was observed in these mice 90 min after OVA challenge, accompanied by increased serum levels of corticosterone.

Not only food allergy, but also other allergic diseases have been associated with neuropsychological sequelae [7,16,17]. Symptoms of developmental and behavioural dysfunction were more frequent in children with asthma compared to control children and asthma severity was shown to correlate with greater behavioural difficulties [16,18]. In addition, ADHD was positively associated with eczema and asthma [19] and a preliminary report indicated that ASD was more prevalent among children with mastocytosis [20], suggesting a role for mast cell activation in triggering neurological manifestations. Preclinical studies showed that OVA-immunized mice challenged via the airways displayed comparable brain activation as mice challenged via the oral route [15]. Furthermore, allergic rhinitis increased anxiety and reduced social interaction in rats and mice, one day after allergen challenge [21].

Despite these clinical and preclinical indications, there is still much debate on the existence of food allergy-enhanced psychosocial disabilities and the question whether food allergy in mice affects social and repetitive behaviour has never been explored. Therefore, this study investigated the effects of a food allergic immune response on social interactions, repetitive behaviour and spontaneous alternation in mice and examined associated region-specific neuronal activation and monoamine levels.

## 2. Materials and methods

### 2.1. Cow's milk allergy mouse model

Three-week-old, specific pathogen free, male C3H/HeOuJ mice were purchased at Charles River Laboratories (L'Arbresle Cedex, France) and housed at the animal facility of the Utrecht University on a 12 h light–dark cycle with access to food and water *ad libitum*. Mice were bred and raised on a cow's milk protein-free diet (Special Diet Services, Witham, UK). All animal procedures were approved by and conducted in accordance with the guidelines of the Animal Ethics Committee of Utrecht University (approval number: DEC2009.I.12.112, DEC2011.I.08.082). After one-week habituation, mice were sensitized intragastrically (i.g.) with 20 mg whey/0.5 mL PBS containing 10 μg cholera toxin (CT, DMV International, Veghel, The Netherlands) as an adjuvant. Sham-sensitized control mice received CT alone. Mice were sensitized once a week for 5 consecutive weeks as previously described by Schouten et al. [22]. One week after the last sensitization, mice were challenged i.g. with 50 mg whey/0.5 mL PBS. The day after challenge, mice were exposed to a social behaviour test. To further exploit behaviour and avoid multiple behavioural testing on one day, self-grooming and T maze alternation was assessed one and two days after the last sensitization, respectively. An intestinal allergic response to the fifth sensitization is confirmed by elevated serum levels of mMCP-1 in whey-sensitized mice (data not shown).

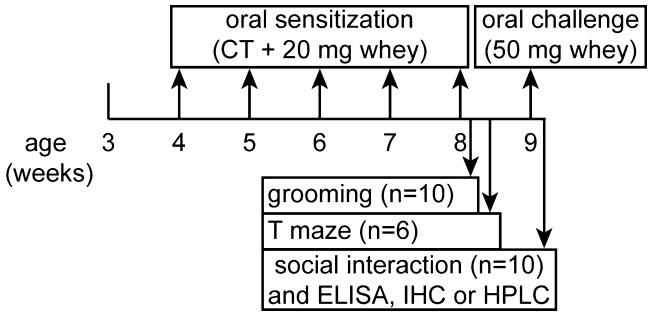


**Fig. 1.** A schematic overview of the sensitization and challenge protocol and the behavioural tests performed. Mice were exposed to one of the behavioural tests; self-grooming (one day after last sensitization), spontaneous alternation in the T maze (two days after last sensitization) or social interaction (one day after challenge). Serum, brain and intestinal tissues of mice that were exposed to the social interaction test were used for further analysis by ELISA, IHC and HPLC.

### 2.2. Social interaction test

The behavioural assessment used was adapted from a previous description [23,24]. The morning after oral challenge, mice were exposed to a social interaction test ($n = 10$ per group). Mice were placed in a 45 × 45 cm open field, with a small perforated Plexiglas cage (10 cm diameter) located against one wall allowing visual, olfactory and minimal tactile interaction (Fig. 1a). Mice were habituated to the open field for 5 min and an age- and gender-matched unfamiliar target mouse was introduced in one of the cages for an additional 5 min. Open fields were cleaned with water followed by 70% ethanol after each test. By using video tracking software (EthoVision 3.1.16, Noldus, Wageningen, The Netherlands), an interaction zone around the cage was digitally determined. Time spent in the interaction zone, latency until first occurrence in the interaction zone and total distance moved was measured.

### 2.3. Self-grooming

The morning after the last sensitization, mice ($n = 10$ per group) were scored for spontaneous grooming behaviours as described earlier [25,26]. Each mouse was placed individually in an empty home cage (35 cm × 20 cm) without bedding and video recordings were used for behavioural scorings of frequency and cumulative time spent grooming all body regions. Open field was cleaned with water followed by 70% ethanol after each test. After a 5 min habituation period in the cage, each mouse was scored blindly for 5 min by two independent researchers. Inter-rater reliability was 97.8%.

### 2.4. T maze spontaneous alternation

Two days after the last sensitization, spontaneous alternation was tested in a T maze set-up. The T-maze was (49 cm long × 10 cm wide × 19 cm high) and two lateral arms (32 cm long × 10 cm wide × 18 cm high). A trial consisted of 2 runs, with a time interval