

Neural correlates of IgE-mediated food allergy

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Abstract

Although many authors have considered the possibility of a direct interaction between food allergy and behavioral changes, the evidence supporting this hypothesis is elusive. Here, we show that after oral ovalbumin (OVA) challenge, allergic mice present higher levels of anxiety, increased Fos expression in emotionality-related brain areas, and aversion to OVA-containing solution. Moreover, treatment with anti-IgE antibody or induction of oral tolerance abrogate both food aversion and the expression of *c-fos* in the central nervous system (CNS). Our findings establish a direct relationship between brain function and food allergy, thus creating a solid ground for understanding the etiology of psychological disorders in allergic patients.

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1. Introduction

Food allergy and other allergic diseases have emerged as major public health problems due to their dramatic increase over the past two to three decades. It is estimated that approximately 8% of children and 2% of the adult population suffer from food allergy (Sampson, 1999; Sampson and Burks, 1996). Additionally, food-induced anaphylaxis is now the leading single cause of anaphylactic reactions treated in hospitals in the USA and UK (Sampson, 2000; Wuthrich and Ballmer-Weber, 2001). Food-induced allergic reactions are responsible for a myriad of symptoms involving the skin, gastrointestinal (GI) tract, and airways (Crowe and Perdue, 1992; Sampson, 1999). In the GI tract, mast cell degranulation (triggered by cross-linking of IgE, bound to its high-affinity receptor) results in an augmentation of ion secretion by the epithelium and an increase in vascular permeability accompanied by contraction of smooth muscle layers and edema of the mucosa (Bischoff et al., 2000; Yu and Perdue, 2001). This

jeopardizes the integrity of the epithelial barrier, thus favoring the penetration of other antigens (Ags) (Bischoff et al., 2000; Yu and Perdue, 2001). Although the cellular and molecular mechanisms underlying food allergy are well understood, little is known about the effect of food allergy on brain function. Some authors have reported an association between food allergy and behavioral changes (Atkins, 1986; Crayton, 1986; Gettis, 1989). Indeed, it has been shown that human patients suffering from food allergy present higher prevalence of trait anxiety and/or depression than nonhealthy controls with lactose intolerance and healthy controls (Addolorato et al., 1998). However, in similar studies, other authors have failed to find this association (Pearson et al., 1983; Peveler et al., 1996), arguing that the diagnosis of psychological symptoms in allergic patients was due to an artifact of referral bias (Peveler et al., 1996). The main argument against allergy-induced psychological symptoms has been the lack of supportive evidence for a direct effect of allergy on neural activity and behavior (Pearson, 1988).

In the present study, we investigated whether food allergy could modulate neural activity and behavior. More specifically, we examined *c-fos* expression in brain areas that are involved in the emotional and affective aspects of adaptive and behavioral responses. For this purpose, a

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mouse model known to induce changes in feeding behavior after immunization with ovalbumin (OVA) was employed. In this model, when a sweetened OVA-containing solution is offered to OVA-immunized or nonimmunized mice, in a two-bottle preference test, the OVA-immunized animals but not the nonimmunized, avoided drinking the OVA-containing solution (Basso et al., 2001; Cara et al., 1994). Here, we show that food aversion of allergic mice is associated with increased levels of anxiety and with increased *c-fos* activity in emotionality-related brain areas, such as the paraventricular nucleus of the hypothalamus (PVN) and the central nucleus of the amygdala (CeA). We also show that induction of immunological tolerance or treatment with non-anaphylactic anti-IgE antibodies block these alterations. This study shows a direct effect of food allergy on brain function and behavior, indicating some pathways by which allergic reactions influence emotionality.

2. Materials and methods

2.1. Animals

Six- to eight-week-old male BALB/c mice were obtained from our colony at the Department of Pathology, School of Veterinary Medicine—USP, São Paulo, Brazil. The animals were housed accordingly in a controlled temperature (22–26 °C) and artificially lighted room on a 12-h-light–12-h-dark cycle (lights on at 7:00 a.m.) with free access to rodent chow and water. Animals were housed and used in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo; these guidelines are similar to those of the National Research Council, USA.

2.2. OVA immunization

Mice were immunized on days 0 and 14 with an intraperitoneal injection of 4 µg grade V chicken egg OVA (Sigma, St. Louis, MO) and 1.6 mg of aluminum hydroxide gel suspended in PBS to a total volume of 0.4 ml.

2.3. Oral Ag administration

Oral tolerance to OVA was induced by offering the animals *ad libitum* a 1% OVA (grade II, Sigma) solution dissolved in drinking water for 5 consecutive days before sensitization (day 0). This solution was freshly prepared each 24 h.

2.4. Anti-IgE antibody treatment

Mice were treated intravenously with 0.25 mg purified rat IgG1 anti-mouse IgE (clone R35-92, Pharmingen, San

Diego, CA) or control rat IgG (Sigma) in a total volume of 0.25 ml, 1 h after the second OVA immunization (day 14).

2.5. Two-bottle preference test

On day 18, mice were housed individually. Each individual cage was supplied with two identical bottles (both filled with water) at opposite sides. Three days later (day 21), one of the bottles was filled with a sweetened (0.5% saccharin) solution of egg white (20%) in water. The other bottle remained filled with water. Every bottle was weighed immediately before the beginning of the aversive test and after 24 h. The total fluid intake (water plus sweetened egg white solution) was expressed as grams of ingested solution. To evaluate their preference for the sweetened solution, we used the following calculation: percentage of preference = sweetened solution of egg white consumption/total fluid intake $\times$ 100.

2.6. Behavioral analysis

Mice were submitted to oral water delivery by gavage for 3 consecutive days preceding day 21. This allowed habituation to the procedure and minimized possible handling- or gavage-induced behavioral changes. On day 21, mice were given 0.5 ml OVA-containing solution (60% egg white solution in water) by gavage. One hour after oral OVA challenge, mice were observed in an open field similar to that described earlier (Palermo Neto et al., 2001). Briefly, the open field consists of a round wooden arena (45 cm diameter, 20-cm-high walls) painted white and with its floor divided in several cells of equal size. Each mouse was individually placed in the center of the apparatus, being observed for 5 min for ambulation frequency (total entries into cells). Behavioral analysis in the plus maze was performed immediately after the end of the open-field test. The plus maze device used was made of wood and had two open arms (15 $\times$ 5 cm) and two enclosed arms of the same size with 15-cm-high walls. The apparatus is elevated 100 cm above the ground and is configured in such way that similar arms are placed opposite each other. Each mouse was placed in the central area of plus maze and allowed 5 min of free exploration. The time spent in open arms and enclosed arms exploration were recorded. The percentage of time spent in the open arms is inversely correlated with level of anxiety in this test.

2.7. Fos immunohistochemistry

Immunohistochemistry was performed on samples collected 90 min after oral OVA challenge. Mice were deeply anesthetized with xylazine and ketamine and immediately perfused intracardially with 25 ml of 0.1 M phosphate-buffered saline (PBS) followed by 100 ml

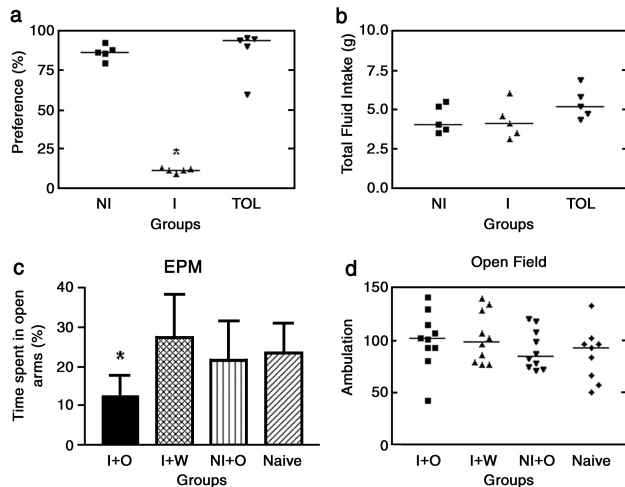


Fig. 1. Behavioral changes in allergic mice. (a) Two-bottle preference test. Preference for the sweetened OVA-containing solution by individual mice shows that while tolerant (TOL) and nonimmunized (NI) mice preferred drinking the antigen-containing solution, immunized (I) animals developed food aversion, choosing water over the sweetened OVA solution. Horizontal bars represent medians. $n=5$ in each group. *Significant difference from all other groups by the Kruskal–Wallis test followed by the Kolmogorov–Smirnov two-sample test ($P<0.05$). (b) Total fluid intake for individual mice show a similar distribution of values for NI, I, and TOL groups. Horizontal bars represent medians. (c) Elevated plus maze test (EPM). One hour after oral challenge, immunized mice receiving OVA (I+O) presented increased levels of anxiety as they spent less time exploring the open arms of the EPM than those that received water (I+W), the nonimmunized mice receiving OVA (NI+O), and the naive mice. $n=10$ in each group. *Significant decrease compared to all other groups by ANOVA followed by post hoc Student–Newman–Keuls test [mean $\pm$ S.D.; $F(3,36)=5.17$, $P<0.05$]. (d) Open field. Numbers of entries in cells of the open field for individual mice show that ambulation frequency was similar among the groups. Horizontal bars represent medians. $n=10$ in each group.

of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Their brains were removed and postfixed for at least 5 h in the same fixative and cryoprotected overnight in 30% sucrose in PB. Coronal, frozen 30- μ m sections were cut and collected in PB. Floating sections were incubated overnight with an antiserum directed against the Fos protein (Ab-5, Calbiochem, San Diego, CA). The Fos antibody was used at 1:1000 in a solution of 5% normal goat serum in PB with 0.3% Triton X-100. After washing, the sections were incubated for 90 min with a biotinylated goat anti-rabbit antibody (Jackson, West Grove, PA) diluted at 1:200. Following incubation with avidin–biotin complex (Vectastain kit, Vector, Burlingame, CA), peroxidase activity was revealed using diaminobenzidine as chromogen and hydrogen peroxide. The sections were mounted on gelatin-coated slides, dehydrated, and coverslipped. In some cases, counterstaining with Giemsa helped the evaluation of the percentage of Fos-positive neurons. Numbers of stained nuclei were estimated using an image analysis

software (Image-Pro® Plus, Media Cybernetics, Silver Spring, MD).

2.8. Determination of OVA-specific IgG1

OVA-specific serum IgG1 antibodies were assayed by sandwich ELISA as described earlier (Russo et al., 2001). Briefly, Nunc-Immuno Plates Maxi-Sorb (Inter-Med, São Paulo, Brazil) were coated overnight at 4 °C with 2 μ g of OVA per well, washed with (PBS)–Tween 0.05%, and blocked with 0.25% casein in PBS. After washing, the serum samples were added and incubated for 1 h. The bound antibodies were revealed by the addition of 1 μ g/ml of goat anti-mouse IgG1 for 1 h followed by washings and the addition of peroxidase-conjugated rabbit anti-goat IgG (H+L) antibodies (Southern Biotechnology, Birmingham, AL) for 1 h. After washings, the reaction was developed by addition of 100 μ l/well of substrate containing 0.5 mg/ml of *o*-orthophenylenediamine dihydrochloride (Sigma) and 0.0015% of H_2O_2 in 0.1 M citric acid/sodium citrate buffer (Merck, São Paulo, Brazil), pH 5.0. The reaction was stopped with 4 M H_2SO_4 and the absorbance of the samples determined at 490 nm using a Multiskan EX (Thermo Labsystems, Vantaa, Finland). The concentrations of IgG1 antibodies were estimated by comparison with IgG1 standards run in parallel on plates coated with rabbit anti-mouse Ig (Southern Biotechnology).

2.9. Determination of OVA-specific IgE

For OVA specific serum IgE determinations, plates were coated overnight at 4 °C with 2 μ g/ml of goat anti-mouse IgE antibodies (Southern Biotechnology). The serum samples were added to the wells followed by biotin-labeled

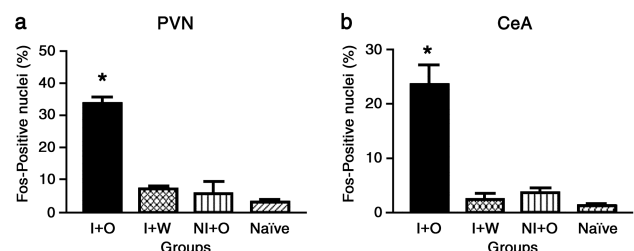


Fig. 2. *c-fos* expression in emotionality-related brain areas of allergic mice. Immunized mice receiving OVA (I+O) presented increased number of Fos-positive nuclei than that observed in immunized animals that received water (I+W), in nonimmunized mice receiving OVA (NI+O), and in naive mice. *c-fos* expression was evaluated 90 min after oral challenge. (a) Paraventricular nucleus of the hypothalamus (PVN). $n=3-4$ in each group. *Significant increase compared to all other groups by ANOVA followed by post hoc Tukey–Kramer test [mean $\pm$ S.D.; $F(3,9)=86.3$, $P<0.01$]. (b) Central nucleus of the amygdala. $n=3-4$ in each group. *Significant increase compared to all other groups by ANOVA followed by post hoc Tukey–Kramer test [mean $\pm$ S.D.; $F(3,9)=81.95$, $P<0.01$].

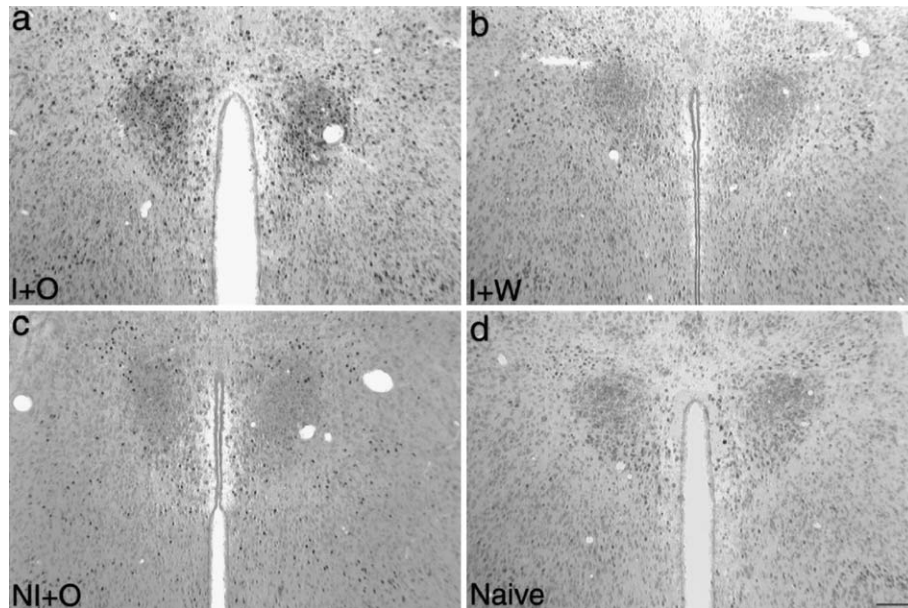


Fig. 3. Food allergy-induced *c-fos* expression in the PVN. Representative brain coronal sections showing intense Fos staining in neurons of the paraventricular nucleus of the hypothalamus (PVN) in immunized mice challenged with an oral dose of OVA solution (a). In contrast, Fos-positive nuclei were almost absent in immunized mice receiving water (b), in nonimmunized animals receiving OVA (c), and in naive mice (d). Counterstained with Giemsa. Scale bar, 100 μ m.

OVA. The bound OVA-biotin was revealed by extraAvidin peroxidase conjugated (Sigma) followed by 100 ml/well of substrate as described above. OVA-specific IgE levels of samples were related to an internal standard obtained from pooled sera of hyperimmunized BALB-c mice that was arbitrarily assigned to be 1000 U.

3. Results

3.1. Allergic mice develop food aversion

We first determined the feeding behavior of allergic animals. For this, we used an experimental model in which

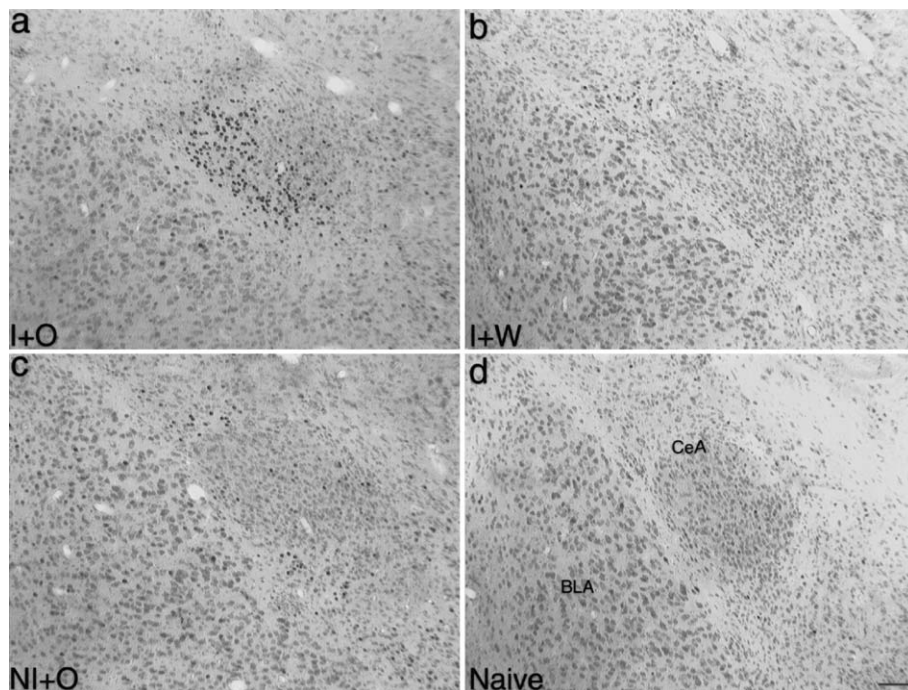


Fig. 4. Food allergy-induced *c-fos* expression in the CeA. Representative brain coronal sections showing intense Fos staining in neurons of the central nucleus of the amygdala (CeA) in immunized mice challenged with an oral dose of OVA solution (a). In contrast, Fos-positive nuclei were almost absent in immunized mice receiving water (b), in nonimmunized animals receiving OVA (c), and in naive mice (d). BLA, basolateral nucleus; CeA, central nucleus. Counterstained with Giemsa. Scale bar, 100 μ m.

nonimmunized or OVA-immunized mice face the choice of drinking either water or a sweetened OVA-containing solution (Cara et al., 1994). We found that nonimmunized animals preferred the sweetened OVA-containing solution while OVA-immunized animals developed food aversion as they preferred drinking water over the sweetened Ag-containing solution (Fig. 1a). It appears that food aversion is triggered by OVA-specific antibodies (Cara et al., 1994). Therefore, we next examined whether the induction of oral tolerance to OVA would block food aversion. It was previously shown that the induction of oral tolerance by exposing mice to OVA in the drinking water before OVA immunization suppresses Th2-associated allergic responses (Russo et al., 2001). We found that mice exposed to 1% OVA dissolved in the drinking water for 5 consecutive days did not develop food aversion. Instead, tolerant mice showed the same preference for the sweetened OVA-containing solution as that presented by nonimmunized animals (Fig. 1a). Moreover, total liquid intake (sweetened OVA solution plus water) did not differ among experimental groups (Fig. 1b), indicating that the reduction of preference for the sweetened OVA-containing solution by immunized mice was due to a decreased intake of the antigen-containing solution and increased water intake.

3.2. Allergic mice present anxiety-like behavior

To further explore the repercussion of food allergy on the central nervous system (CNS), we examined the anxiety-like behavior of allergic mice. OVA-immunized mice were challenged by an oral dose of OVA and observed 1 h later in an open field, and subsequently, in an elevated plus maze (EPM). The EPM is a validated behavioral model widely employed for the quantitative analysis of anxiety in rats and mice (Lister, 1987; Pellow et al., 1985). Rodents naturally avoid open areas, and drugs or anxiogenic stimuli induce even shorter time of exploration of the open arms of this arena (Lister, 1987; Pellow et al., 1985). Oral OVA delivery to immunized mice induced a clear anxiogenic effect (Fig. 1c). Thus, we observed that mice from the I+O group (immunized receiving OVA) spent less time exploring the open arms of the EPM than those that received water (I+W) than nonimmunized mice receiving OVA solution (NI+O) and naive mice (Fig. 1c). No difference was detected between naive animals and those from I+W or NI+O groups (Fig. 1c), showing that the anxiogenic effect observed was not induced by forced ingestion itself. Furthermore, oral presentation of the antigen to immunized mice did not interfere with ambulation in the open field, which is important when interpreting data generated in the EPM (Fig. 1d).

3.3. Allergic mice present increased *c-fos* expression in emotionality-related brain areas

We were interested in determining which brain areas could be associated with the behavioral changes observed

in our model. Since it is well established that *c-fos* expression is a good marker of neuronal activation (Dragunow and Faull, 1989; Sagar et al., 1988), we determined the number of Fos-positive cells in different brain areas. We found that allergic mice had an increased *c-fos*

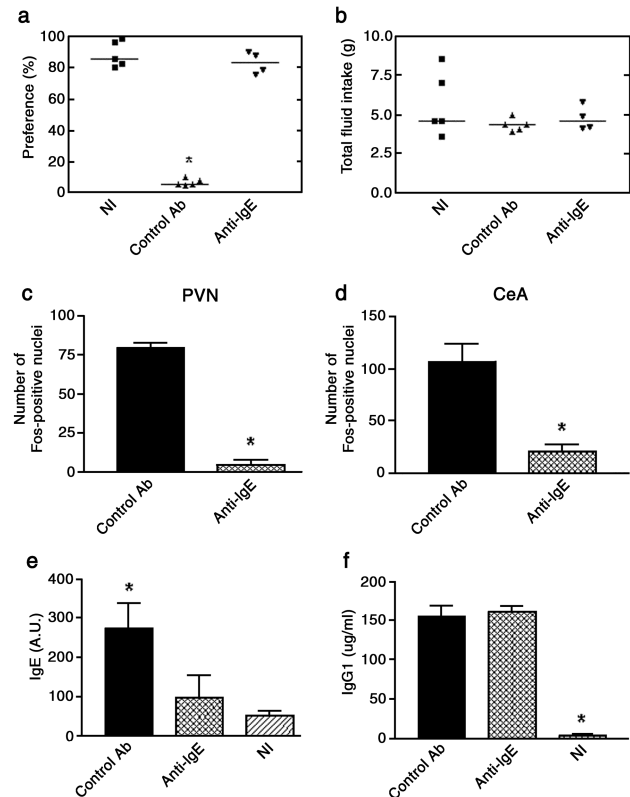


Fig. 5. OVA-specific IgE plays a fundamental role on food allergy-induced behavioral changes and *c-fos* expression. (a) Two-bottle preference test. Preference for the sweetened OVA-containing solution by individual mice show that while nonimmunized (NI) mice and animals treated with anti-IgE antibody (Anti-IgE) preferred drinking the antigen-containing solution, those treated with the control antibody (Control Ab) developed food aversion, choosing water over the sweetened OVA solution. Horizontal bars represent medians. $n=4-5$ in each group. *Significant difference from all other groups by the Kruskal–Wallis test followed by the Kolmogorov–Smirnov two-sample test ($P<0.05$). (b) Total fluid intakes for individual mice show a similar distribution of values for NI, Control Ab, and Anti-IgE groups. Horizontal bars represent medians. (c) While mice treated with the control antibody (Control Ab) presented intense Fos staining in neurons of the PVN and CeA (d), few Fos-positive nuclei were observed in animals treated with anti-IgE antibody. $n=3$ in each group. *Significant decrease compared to Control Ab group by the unpaired Student's *t*-test ($P<0.05$). Data represent mean $\pm$ S.D. (e) Treatment of immunized mice with anti-IgE antibody blocked the appearance of OVA-specific IgE in the serum. While mice treated with the Control Ab presented high levels of IgE, those treated with anti-IgE antibody presented levels similar to that observed in nonimmunized animals (NI). $n=3$ in each group. *Significant difference from all other groups by ANOVA followed by post hoc Tukey–Kramer test [mean $\pm$ S.D.; $F(2,10)=25.71$, $P<0.01$]. (f) Treatment of immunized mice with anti-IgE antibody did not interfere with OVA-specific IgG1 production. Mice treated with anti-IgE or with the control antibody presented similar levels of serum IgG1. *Significant difference from all other groups by ANOVA followed by post hoc Tukey–Kramer test [mean $\pm$ S.D.; $F(2,10)=575.51$, $P<0.01$].

expression mainly in the paraventricular nucleus of the hypothalamus (PVN) and in the central nucleus of the amygdala (CeA). Interestingly, these areas are involved in the generation and control of adaptive responses endowed with emotional or affective contents (Adolphs et al., 1995; Monnikes et al., 1992; Morton et al., 1989). In OVA-immunized animals, we detected a high number of Fos-positive neurons 90 min after OVA challenge in both areas (PVN, Figs. 2a and 3a; CeA, Figs. 2b and 4a). In contrast, Fos-positive neurons were virtually absent in OVA-immunized animals that received water (I+W) (Figs. 3b and 4b), in nonimmunized mice that received OVA (NI+O) (Figs. 3c and 4c), and in naive animals (Figs. 3d and 4d). The presence of nutrients in the intestinal lumen appears to be sufficient to cause CNS stimulation (Raybould, 2002), and therefore, the activation of brain areas described here could be caused simply by ingestion of the antigen-containing solution. Nonetheless, the fact that Fos staining in nonimmunized animals receiving OVA solution (NI+O) was similar to that seen in naive mice and in immunized animals receiving water (I+W) (Fig. 2a and b) does not support this hypothesis.

3.4. The neural correlates of food allergy are IgE dependent

Since oral tolerance prevented the development of food aversion and also suppressed IgE production (data not shown), we asked whether the allergy-induced behavioral changes and increased Fos expression could be attributed to

OVA-specific IgE antibodies. To test this hypothesis, we treated the OVA-immunized animals with a non-anaphylactic anti-IgE antibody before OVA challenge and analyzed the development of food aversion and the activation of the brain areas described above. We found that treatment with anti-IgE antibody 7 days before oral OVA challenge prevented food aversion. Thus, while animals treated with control rat IgG developed aversion to the sweetened OVA solution and preferred drinking water, those treated with anti-IgE antibody behaved exactly as nonimmunized mice (NI), choosing the sweetened antigen-containing solution over water (Fig. 5a). Again, the total liquid consumption among different experimental groups did not differ (Fig. 5b). Additionally, anti-IgE treatment markedly reduced activation of emotionality-related brain areas, evidenced by the fact that few Fos-positive neurons were observed in the PVN and CeA of mice treated with anti-IgE (Fig. 5c and d). In contrast, animals that received control (rat IgG) antibodies showed intense Fos staining in neurons of the PVN (Figs. 5c and 6a) and CeA (Figs. 5d and 6b). As expected, treatment with anti-IgE antibody blocked the appearance of OVA-specific IgE antibodies (Fig. 5e). In anti-IgE-treated animals, the level of IgE antibodies was similar to that obtained from nonimmunized animals (NI) (Fig. 5e). We have also demonstrated that anti-IgE treatment did not affect the production of OVA-specific IgG1 antibodies (Fig. 5f). Our results clearly show that IgE antibodies are essential for the development of food aversion and for the activation of the emotionality-related brain areas.

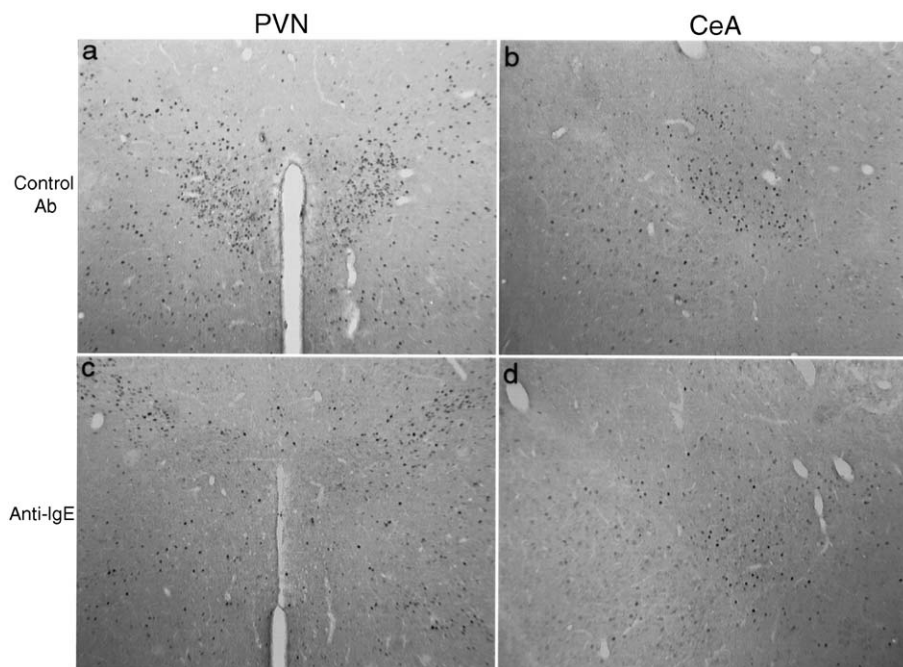


Fig. 6. IgE is essential for food allergy-induced *c-fos* expression in the brain. Representative brain coronal sections showing intense Fos staining in neurons of the paraventricular nucleus of the hypothalamus (PVN) (a) and the central nucleus of the amygdala (CeA) (b) in immunized mice treated with the control antibody (Control Ab). In contrast, few Fos-positive nuclei were observed in the PVN (c) and CeA (d) of mice treated with anti-IgE antibody. Scale bar, 100 μ m.

4. Discussion

Previous reports established a solid biochemical and molecular basis for a bidirectional communication between the immune and neuroendocrine systems (Besedovsky and del Rey, 1996; Blalock, 1989). It is known that the nervous, immune, and endocrine systems share a myriad of receptors for cytokines, neurotransmitters, hormones, and neuropeptides. Additionally, molecules previously reported as specific products of one of the systems have also been demonstrated to exist in the others. For instance, cytokines can be synthesized in the CNS, while hormones, such as ACTH and TSH, can be produced by lymphoid cells (Besedovsky and del Rey, 1996). These bidirectional interactions play an important role not only in the physiology of these systems, but also in several pathological processes (Ader et al., 1995).

In the present work, we focused on the relationships between food allergy and changes in CNS activities involved in emotional or affective behavior. Our results demonstrate a direct relationship between food allergy and changes in CNS activities associated with the expression of behavioral responses such as anxiety and aversion.

We first demonstrated that OVA-immunized mice avoid drinking an antigen-containing solution, which indicates that allergy can modulate feeding behavior. These results confirm previous findings (Basso et al., 2001; Cara et al., 1994) and support the notion that allergic responses influence diet selection. Thus, albeit attracted by its sweet flavor, OVA-immunized mice develop an aversion to OVA-containing diet and avoid it henceforth. However, the induction of OVA-specific unresponsiveness by oral tolerance abrogated the development of food aversion.

The development of food aversion prompted us to study further behavioral alterations and changes in the CNS activity that could be associated with the allergic response. To do that we analyzed in allergic mice the occurrence of anxiety-like behavior and *c-fos* expression in specific brain areas. We found that, after oral OVA challenge, OVA-immunized mice presented increased levels of anxiety because they spent less time exploring the open arms of the EPM. High levels of anxiety and development of food aversion are indicative of emotional or affective responses. Indeed, OVA-immunized mice presented enhanced Fos immunoreactivity in the PVN and CeA after oral OVA challenge. These areas are known to be linked with emotional- and affective-related behavioral patterns. Both the PVN and the CeA are among the main areas of the CNS containing corticotropin-releasing factor (CRF)-expressing neurons. CRF is considered one of the major regulators of adaptive stress responses (Gray, 1993; Gray and Bingaman, 1996; Swanson et al., 1983). It has been shown that socially defeated rats placed on the EPM spent less time exploring the open arms and that this response was reversed by a CRF antagonist (α -helical CRF_{9–41}) injected bilaterally into the CeA (Heinrichs et al., 1992). Likewise, studies in which the

CRF neurons of the CeA and PVN were immunotargeted and eliminated provide clear evidence for their relevance in the expression of emotionality-related behavior in stressed rats exposed to the EPM (Menzaghi et al., 1993). Activation of the CeA and PVN has also been detected in animals submitted to a model of conditioned taste aversion. In this model, intraperitoneal injection of lithium chloride, which is used as unconditioned stimulus, paired with saccharin (the conditioned stimulus) causes an increased expression of *c-fos* in the CeA (Yamamoto et al., 1997) and PVN (Lamprecht and Dudai, 1995). The importance of CeA activation in this model was established because microinjection of oligonucleotide antisense to *c-fos* into this area resulted in a deficient acquisition of conditioned taste aversion (Lamprecht and Dudai, 1996). Moreover, stimulation of T lymphocytes with staphylococcal enterotoxin B results in an increase in CRF mRNA transcripts in these areas (PVN and CeA) (Kusnecov et al., 1999), *c-fos* expression in the PVN (Goehler et al., 2001), and the expression of emotionality-related behavioral patterns (Kusnecov et al., 1999). Early immune events elicited by peripheral immunization with the T-cell-dependent antigen Keyhole limpet hemocyanin (KLH) have also been shown to induce increased *c-fos* expression in the PVN and CeA (Pacheco-Lopez et al., 2002).

Altogether, studies using animal models, as well as clinical studies in human patients (Morris et al., 1998; Raadsheer et al., 1994), clearly point out the importance of these areas in the generation and modulation of affective responses. Although activation of specific rat brain nuclei after intestinal anaphylaxis has been reported by others (Castex et al., 1995), to our knowledge, this is the first study demonstrating evidence of a direct influence of an allergic reaction to dietary antigens on the development of behavioral responses and CNS alterations compatible with elaborate psychological symptoms.

We also elucidated the triggering mechanism of allergy-induced activation of the CNS and development of food aversion. It is well established that allergic hypersensitivity proceeds through two distinct but related phases: an immediate phase which is mediated by IgE and mast cells and a late phase that is mediated by Th2 cells and eosinophils (Sengoku et al., 2001). Because the expression of anxiety-like behavior and activation of PVN and CeA occurred, respectively, 60 and 90 min after OVA challenge, we suspected that these responses were related to the immediate phase of the allergic reaction. To test this hypothesis, we first blocked OVA-specific IgE and IgG1 production by the induction of oral tolerance. As expected, tolerant mice did not develop food aversion. Although oral tolerance also suppresses late-phase reaction (Russo et al., 2001), the experiments with anti-IgE treatment clearly indicate that food aversion and activation of emotionality-related brain areas are triggered by an IgE-dependent mechanism. Thus, by blocking OVA-specific IgE response, we prevented activation of the PVN and CeA in immu-

nized mice and precluded the aversion to the antigen-containing diet.

It follows that IgE-induced mast cell degranulation is likely to be the major mechanism for food allergy signaling to the brain. Since mast cells are distributed along blood vessels of the intestinal mucosa in intimate contact with substance P-positive nerve fibers (C-type fibers) (Stead et al., 1989; Stead et al., 1987), we hypothesized that mediators released by mast cell degranulation could stimulate the nerve endings of the C-fibers that, in turn, would transmit the sensory information to the CNS. Previous evidence that destruction of C-fibers by capsaicin treatment during neonatal period attenuates food aversion supports this hypothesis (Basso et al., 2001). The cross-talk between C-sensory nerve fibers and mast cells has been shown to influence the intestinal smooth muscle contraction and epithelial ion secretion (Yu and Perdue, 2001). In addition, it has been demonstrated that when in coculture, neurons can depolarize following mast cell activation with anaphylactic anti-IgE antibody or compound 48/80 (Janiszewski et al., 1990). Although our data indicate an important role of mast cells on the neural correlates of food allergy, the mediators involved and the molecular mechanisms are still to be determined.

Employing an experimental model designed to explore interactions between the immune and nervous systems, we demonstrated a clear, direct effect of food allergy on behavior patterns, as well as related changes in the CNS. Furthermore, we elucidated the triggering mechanism of these allergy-induced alterations. Our data provide valuable insight for future studies aimed at understanding the development of psychological disorders in allergic patients.

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