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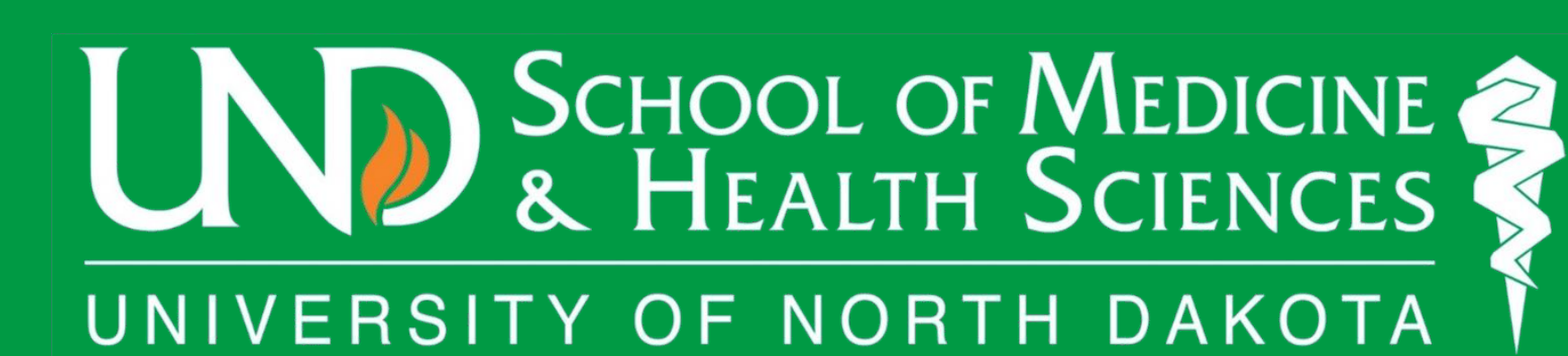
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Continuous consumption of whey protein affects motor and cognitive functions in a mouse model of cow's milk hypersensitivity

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Abstract

Hypersensitization to a food allergen can occur in the absence of immediate allergic reactions, a condition referred to as asymptomatic sensitization. Using mice asymptotically sensitized to a bovine whey allergen, β -lactoglobulin (BLG, Bos d 5), we have previously shown that chronic exposure to the allergen results in behavior changes with increased neuroinflammation and cortical demyelination. However, whether allergen avoidance can resolve these pathologies is unknown. Thus, we aimed to compare immunological and behavioral changes in sham- or BLG-sensitized mice with or without the removal of dietary whey after the mice had consumed either a control diet (CTL) or a whey-protein-containing diet (WP) for 2 weeks. As previously reported, BLG-sensitized mice did not display overt physical reactions during the 2-week allergen exposure period, while their plasma levels of BLG-specific IgE and IgG1 were elevated compared to the respective sham groups. Following an additional 2 weeks on either the CTL or WP diet, these antibodies remained elevated in some of the sensitized mice in both diet groups. The grip-strength test indicated that sham mice that switched to the CTL diet after the 2-week WP diet increased limb strengths. In contrast, BLG-sensitized mice on either diet, as well as sham mice that stayed on the WP diet, showed a decline in limb strengths, suggesting that WP consumption, whether during sensitization or as part of the daily diet, affected their motor function. Furthermore, removing dietary WP improved spatial memory performance with the cross-maze test in both sham and BLG-sensitized mice. These results suggested that continuous exposure to a dietary allergen may influence motor and cognitive functions.

Introduction

We previously showed that C57BL/6J mice sensitized to β -lactoglobulin (BLG: Bos d 5) exhibited anxiety-like and depression-like behavior and neuroinflammation, despite the lack of overt anaphylaxis upon allergen exposure (1, 2).

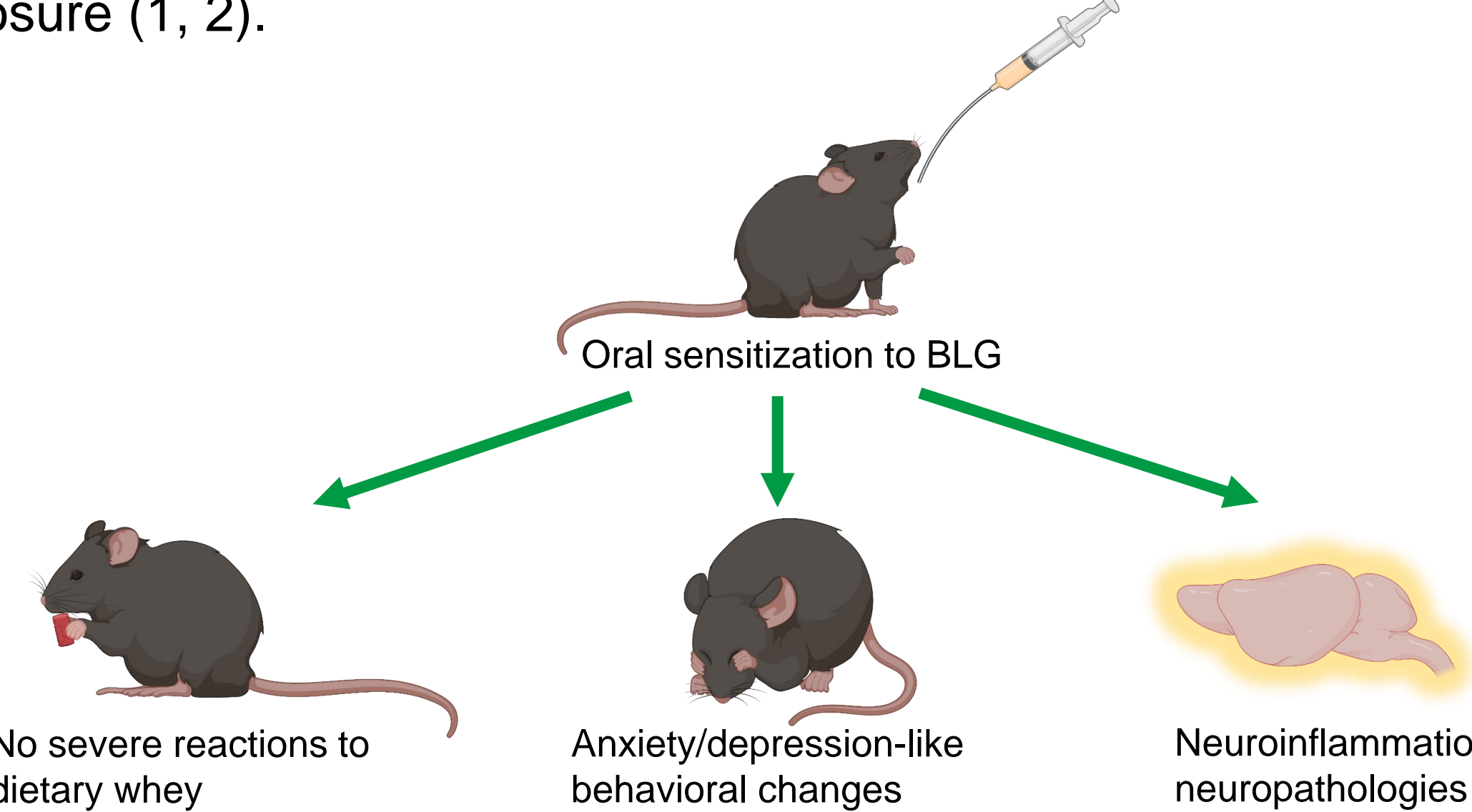


Fig 1. Previous findings in our CMA mouse model.

Hypothesis

Inflammatory immune responses and behavior changes observed in BLG-sensitized mice after the initial allergen exposure would be resolved with allergen avoidance.

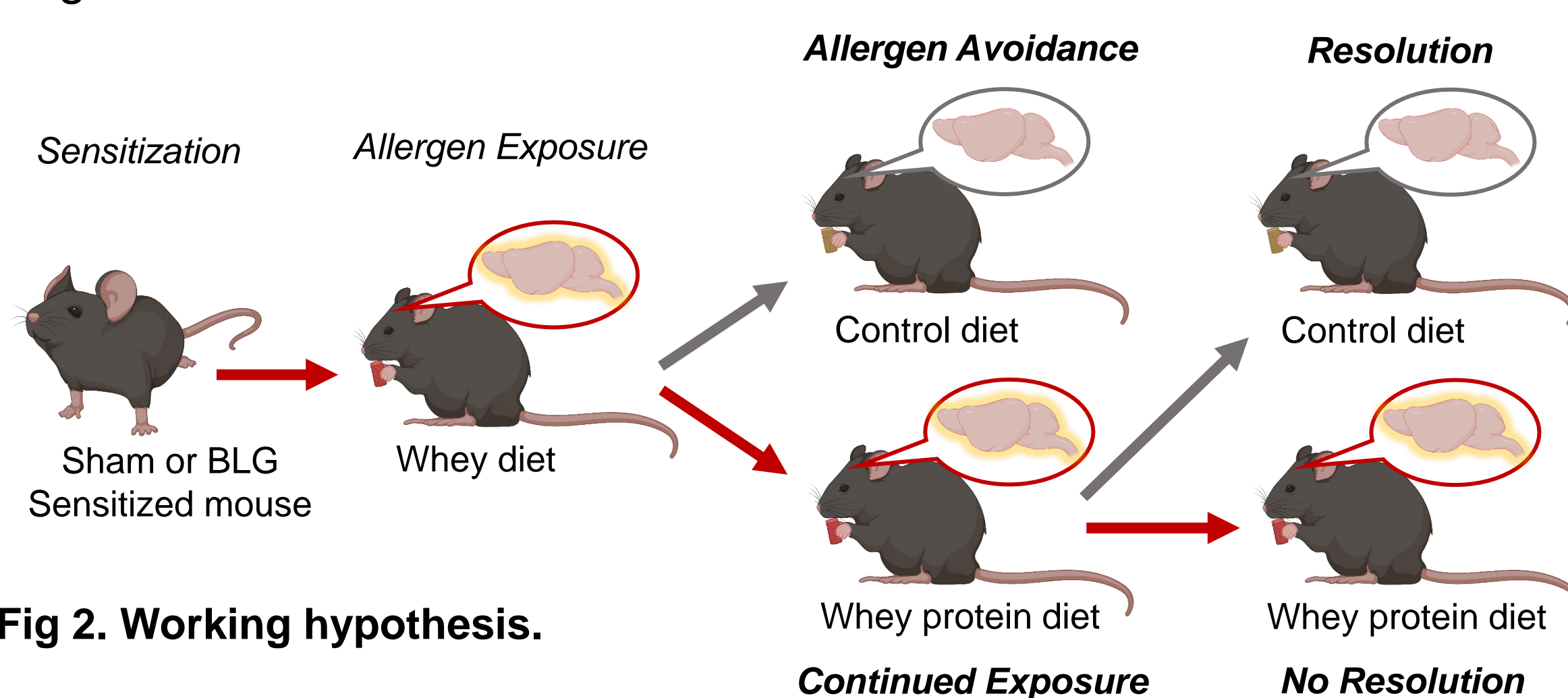


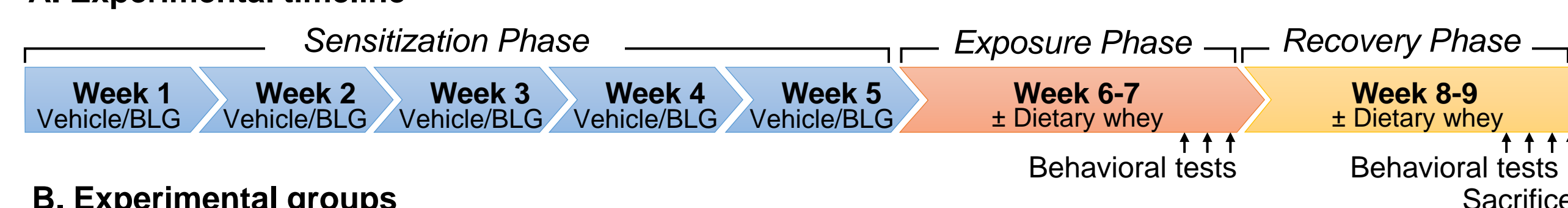
Fig 2. Working hypothesis.

Methods

Allergen sensitization, exposure, and avoidance: Four-week-old C57BL/6J male mice were given five weekly gavage doses of a carbonate buffer (pH 9.0) containing 10 μ g cholera toxin (adjuvant) without or with 1 mg BLG to generate sham or BLG-sensitized mice, respectively. All mice were subsequently placed on a whey-protein (WP) diet for 2 weeks as the allergen exposure phase and either stayed on the WP diet or were switched to a WP-free control (CTL) diet for additional 2 weeks as the recovery phase. All procedures involving mice were approved by UND IACUC prior to the experiments.

Behavior assessments: The all-limb grip strength test and the cross-maze test were performed to assess the animals' motor and cognitive functions (2). All mice were sacrificed one day after the completion of all behavior tests, and blood and brain samples were collected.

A. Experimental timeline



B. Experimental groups

Treatment Phases	C57BL/6J Male Mice			
Sensitization Phase (Week 1-5)	Sham (Vehicle Only)		BLG (1 mg BLG)	
Exposure Phase (Week 6-7)	WP	WP	WP	WP
Recovery Phase (Week 8-9)	CTL	WP	CTL	WP

Fig 3. Experimental design. (A) The experimental timeline of the sensitization, exposure, and recovery phases. (B) Experimental groups showing specified diets fed during the recovery phase.

Cytokine/chemokine assay: The Q5 RayBiotech Quantibody[®] cytokine/chemokine array was used to determine inflammatory changes in the midbrain region.

Immunohistochemistry. Formaldehyde-fixed brains were cryosectioned at 40 μ m and immunostained with an antibody against CD45 (1:500) to detect immune cells.

Results

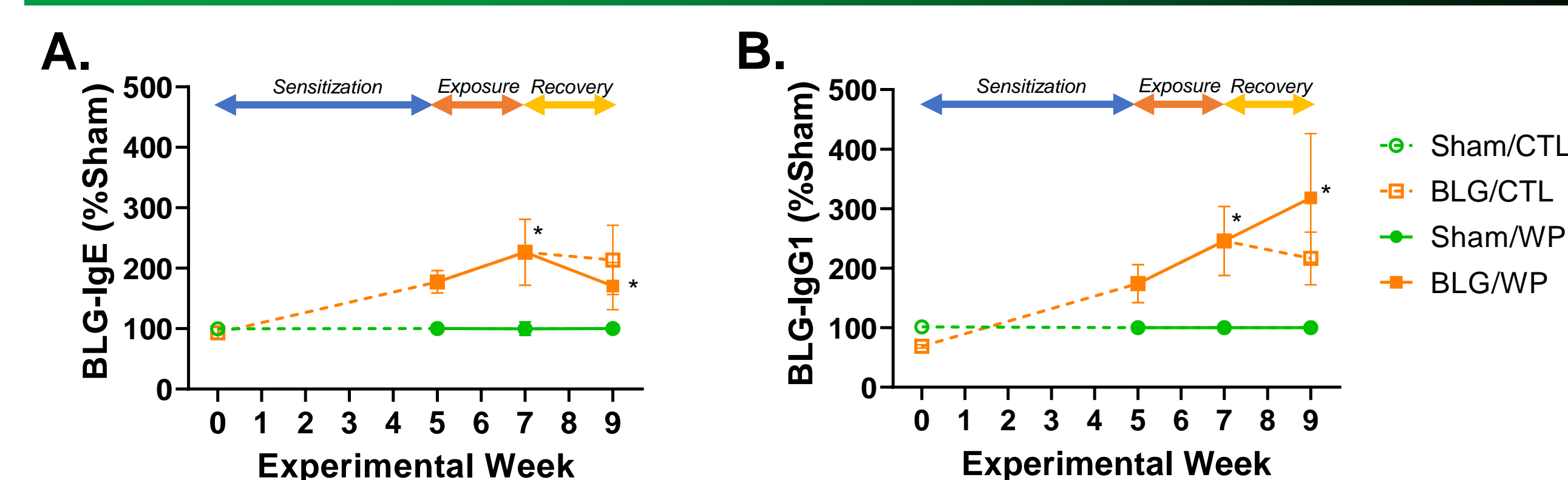


Fig 4. Continued allergen consumption after a 2-week allergen exposure decreased BLG-specific IgE while further increasing IgG1 in sensitized mice. (A) BLG-specific IgE and (B) IgG1 levels were significantly elevated in the plasma of BLG-sensitized mice after the exposure phase. The presence of continued dietary whey protein (WP) differentially altered these immunoglobulin levels. Plasma samples were collected before the start of the study (Week 0) and at the end of the sensitization phase (Week 5), exposure phase (Week 7), and recovery phase (Week 9). Mean \pm SEM (n=7-8), * p <0.05; 2-way ANOVA.

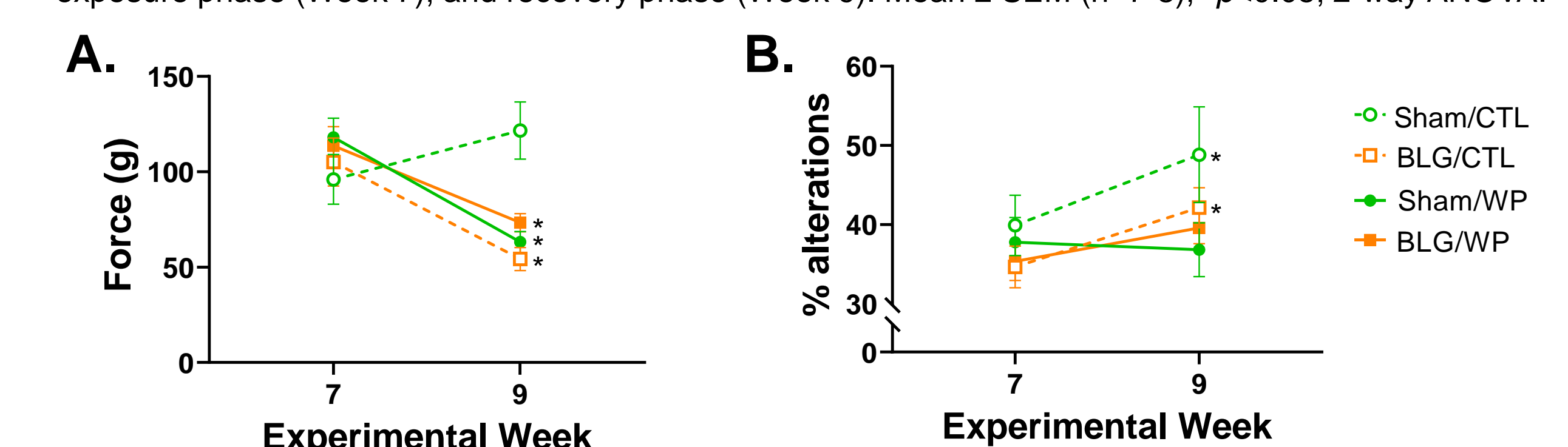


Fig 5. Allergen consumption differentially affects the motor and cognitive functions of sham and BLG-sensitized mice. (A) The grip-strength test showed declines in limb strengths of BLG-sensitized mice on either diet and sham mice that stayed on the WP diet. (B) The cross-maze test indicated that the spatial memory of sham and BLG-sensitized mice significantly improved with allergen avoidance. Mean \pm SEM (n=7-8), * p <0.05 shown for comparisons done at weeks 7 and 9 within the group; 2-way ANOVA.

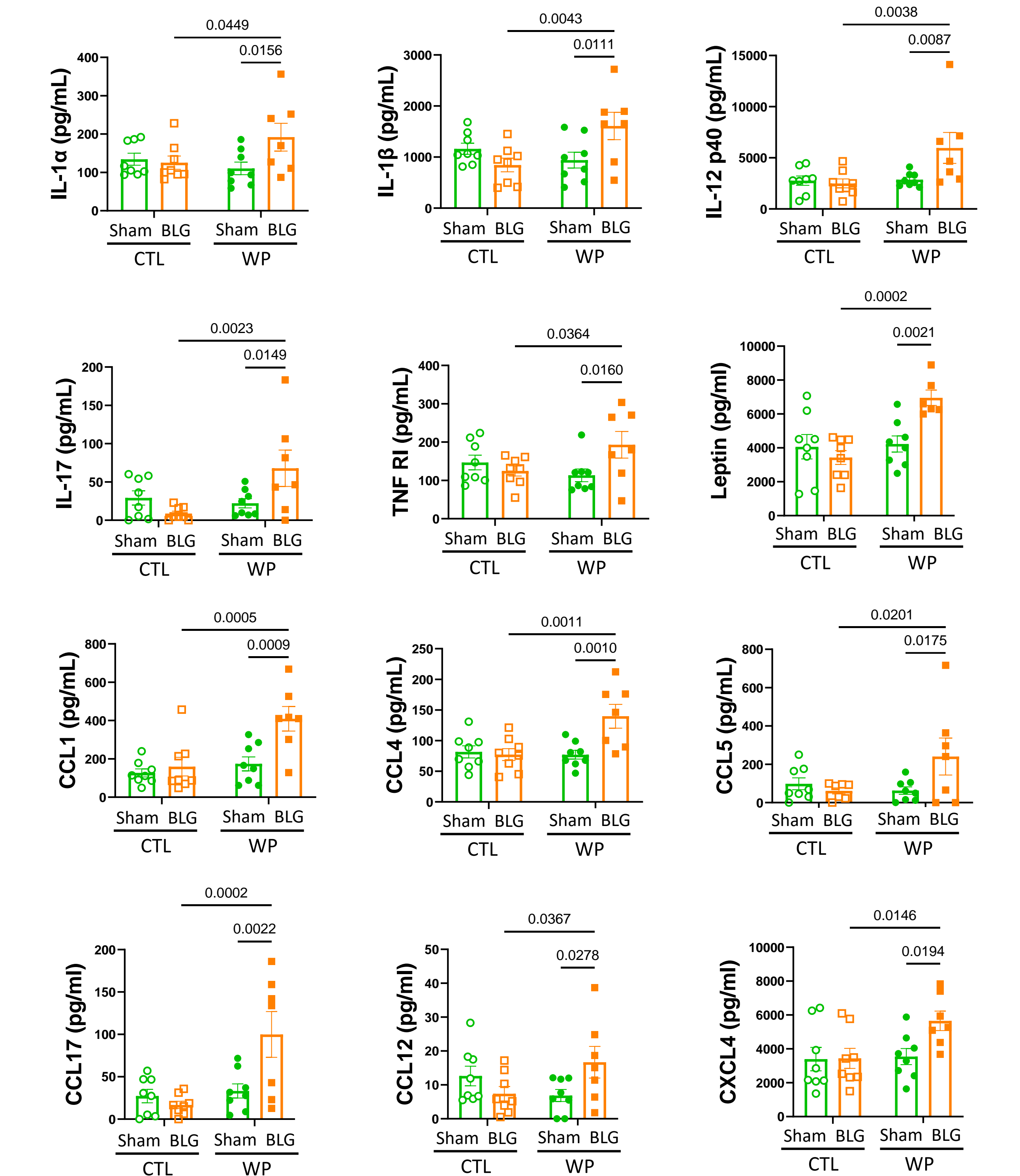


Fig 6. Continued allergen consumption increases various cytokines in the midbrain region of BLG-sensitized mice. Blood samples collected after the recovery phase were assayed to determine the levels of pro-inflammatory cytokines and chemokines quantified using a cytokine/chemokine array. Mean \pm SEM (n=7-8), 2-way ANOVA. Significant p-values are shown.

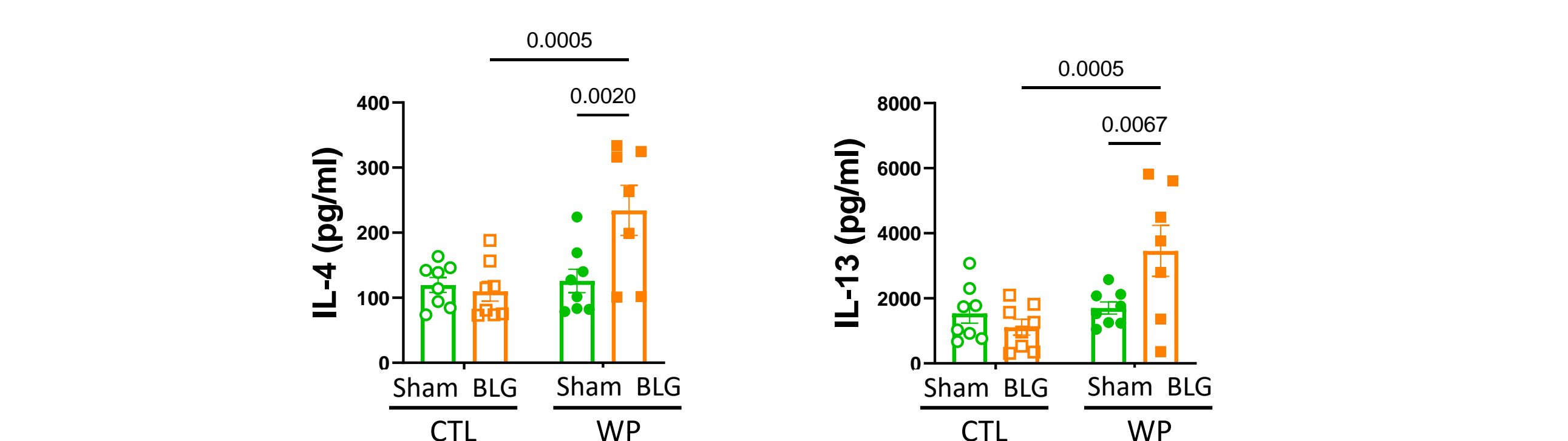


Fig 7. Continued allergen consumption increases allergy-associated cytokines in the midbrain region of BLG-sensitized mice. The levels of IL-4 and IL-13, hallmarks of allergy (3), were quantified using a cytokine array. Mean \pm SEM (n=7-8), 2-way ANOVA. Significant p-values are shown.

Results (cont'd)

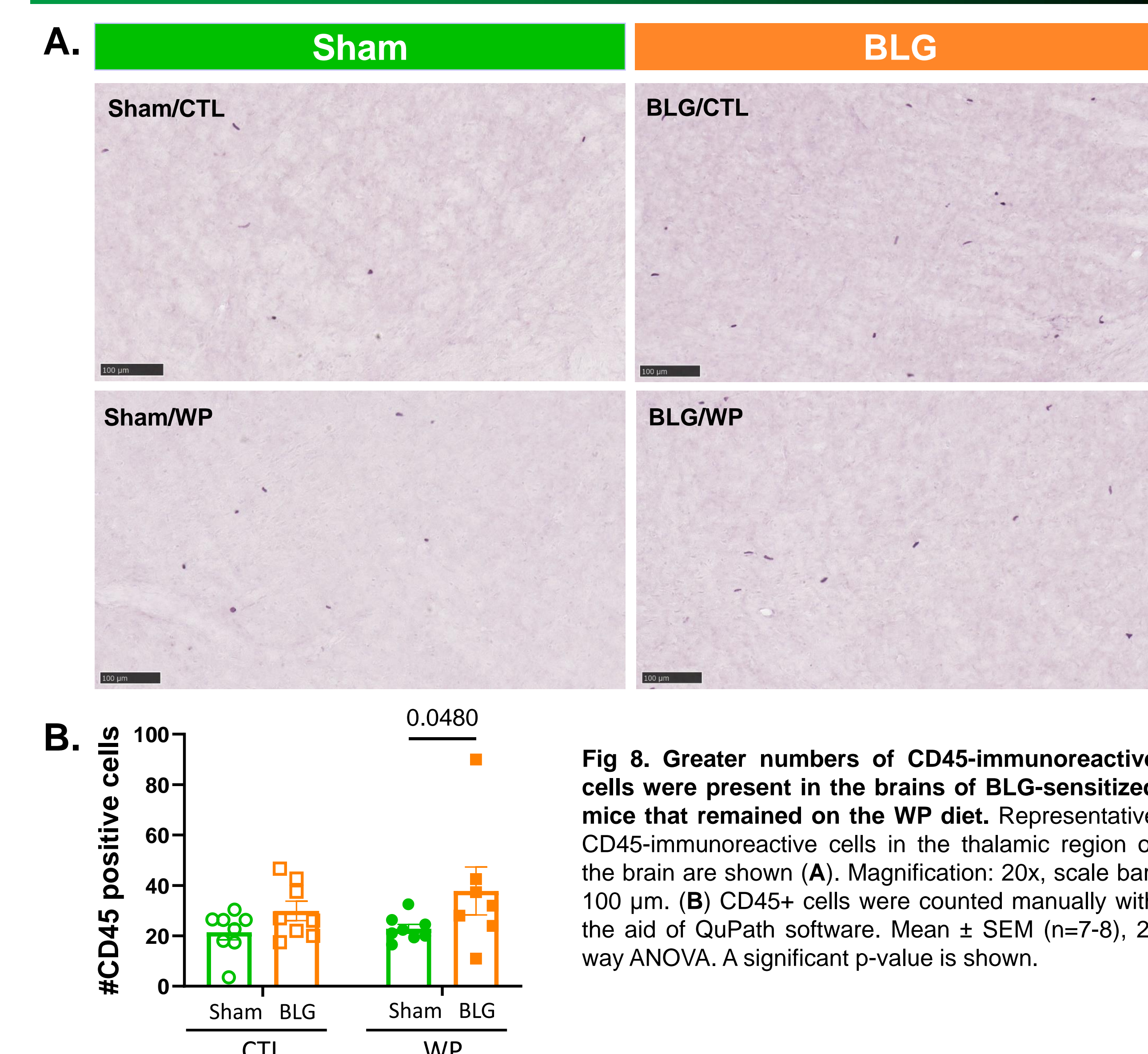


Fig 8. Greater numbers of CD45-immunoreactive cells were present in the brains of BLG-sensitized mice that remained on the WP diet. Representative CD45-immunoreactive cells in the thalamic region of the brain are shown (A). Magnification: 20x, scale bar: 100 μ m. (B) CD45+ cells were counted manually with the aid of QuPath software. Mean \pm SEM (n=7-8), 2-way ANOVA. A significant p-value is shown.

Conclusions & Discussions

- BLG-specific IgE and IgG1 levels remained relatively high in BLG-sensitized mice compared to the respective sham groups even after the allergen was removed from their diet. IgG1 levels further increased with continued consumption of WP, while IgE showed a decline suggestive of desensitization (2).
- Continued consumption of the WP diet negatively influenced motor and cognitive functions in both sham and BLG mice. Sham mice that were placed on the CTL diet during the recovery period showed improvement, supporting that allergen avoidance could be beneficial for these functional modalities.
- The levels of inflammatory mediators in the brains of BLG-sensitized mice were elevated after continued consumption of the WP diet, indicating that chronic allergen exposure maintains neuroinflammatory conditions. Increased numbers of leukocytes in these mice may contribute to maintaining elevated cytokine and chemokine levels.

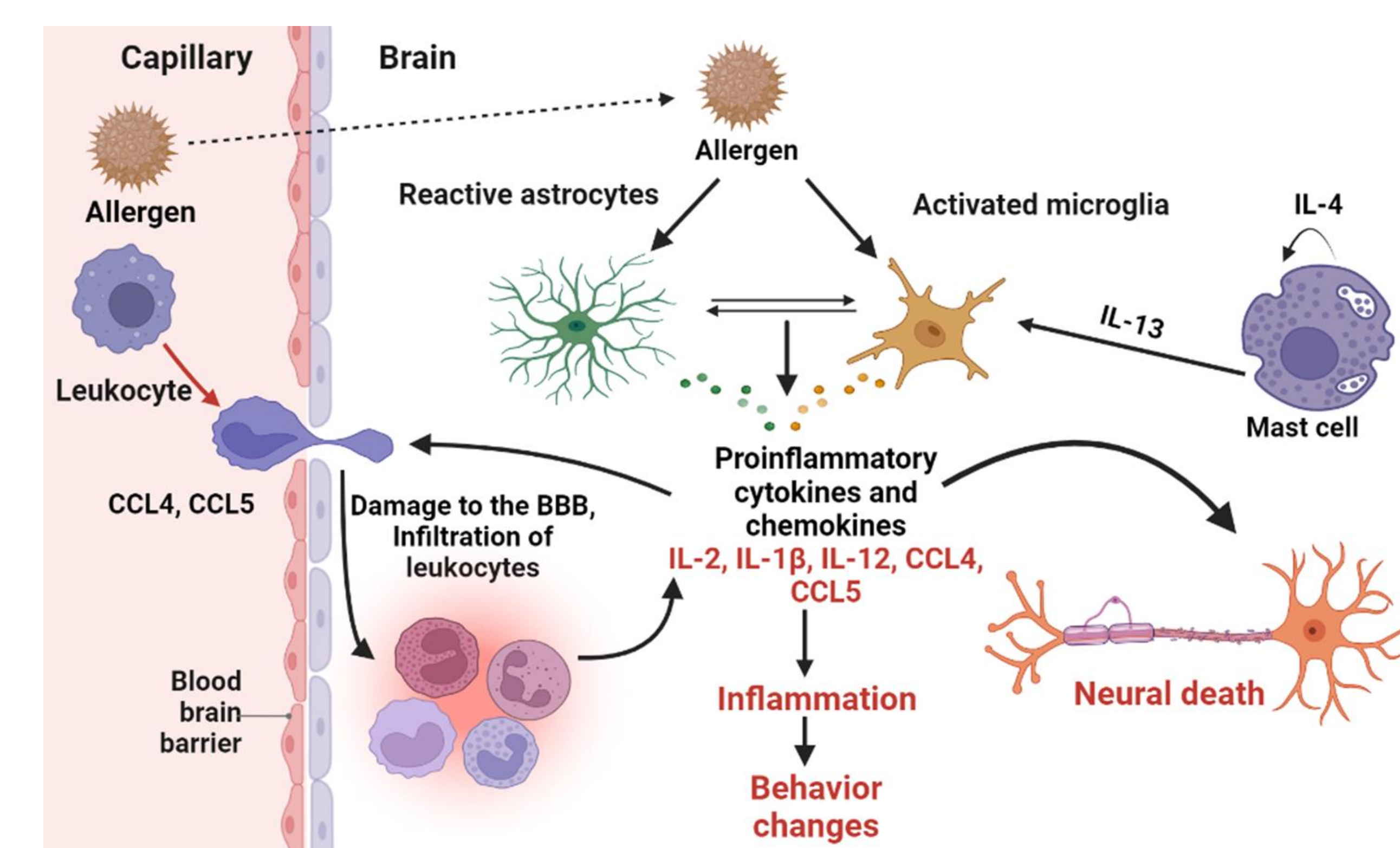


Fig 9. Proposed cellular events leading to neuroinflammation in BLG-sensitized mice with allergen exposure. The involvement of the astrocytes, microglia, and leukocytes in the production of inflammatory cytokines and chemokines is depicted. Infiltration of allergens from the circulation and recruitment of leukocytes across the leaky blood-brain barrier likely maintain neuroinflammation when offending allergens are present in the diet. The resulting increases in proinflammatory mediators may cause neuropathologies that contribute to behavior changes.

References

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Acknowledgements

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