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Loss of APP in mice increases thigmotaxis and is associated with elevated brain expression of IL-13 and IP-10/CXCL10

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that leads to memory loss and is often accompanied by increased anxiety. Although AD is a heterogeneous disease, dysregulation of inflammatory pathways is a consistent event. Interestingly, the amyloid precursor protein (APP), which is the source of the amyloid peptide Aβ, is also necessary for the efficient regulation of the innate immune response. Here, we hypothesize that loss of APP function in mice would lead to cognitive loss and anxiety behavior, both of which are typically present in AD, as well as changes in the expression of inflammatory mediators. To test this hypothesis, we performed open field, Y-maze and novel object recognition tests on 12–18-week-old male and female wildtype and App^{KO} mice to measure thigmotaxis, short-term spatial memory and long-term recognition memory. We then performed a quantitative multiplexed immunoassay to measure levels of 32 cytokines/chemokines associated with AD and anxiety. Our results showed that App^{KO} mice, compared to wildtype controls, experienced increased thigmotactic behavior but no memory impairments, and this phenotype correlated with increased IP-10 and IL-13 levels. Future studies will determine whether dysregulation of these inflammatory mediators contributes to pathogenesis in AD.

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Autor Contribution statement

K Mayagoitia and S. Soriano conceptualized and designed experiments. K.M., S.D.S., S. Shammi, A.J.T., A.A., J.A.M., C.G.W., D.L.B., and J.D.F., performed experiments. K.M., A.J.T., J.A.M., D.L.B., J.D.F., S. Shammi, A.A., and S.S. analyzed the data. K.M. and S.S. wrote the manuscript. All authors have approved the final version of this article.

Ethics approval

All animal experiments were reviewed and approved by Loma Linda University Institutional Animal Care and Use Committee.

Competing interests

The authors declare that they have no competing interests.

Keywords

Anxiety; IL-13; IP-10; Amyloid Precursor Protein; Hippocampus

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative condition that leads to memory loss and is often accompanied by increased anxiety [1]. AD pathophysiology includes amyloid plaques and neurofibrillary tangles, and chronic inflammation. It is believed that inflammation can precede disease outbreak since aberrant inflammatory responses are evident years before the clinical onset of AD [2, 3]. In addition, genome-wide association studies with AD populations identified significant overrepresentation of association signals in pathways associated with the immune response [4]. Interestingly, though the amyloid precursor protein (APP) is the source of the amyloid plaque component Aβ peptide, current evidence suggests a more nuanced biological function for APP [5, 6]. APP is a multifunctional protein necessary for the efficient regulation of the innate immune response against a range of stress stimuli. For example, APP protects against inflammation in the brain in a mouse model of Niemann–Pick disease type C [7]. Also, App^{KO} mice displayed aberrant innate immune cell responses to a range of inflammatory stimuli [8], and reactive gliosis is evident as early as 14 weeks of age [9]. Furthermore, APP is a potent antimicrobial/viral agent, inhibiting the growth of pathogens and replication of influenza virus A via its cleaved product Aβ [10, 11]. These observations strongly point to APP as a protective molecule in the CNS and a regulator of the innate immune response and raises the possibility that loss of this function may lead to deleterious effects in cognition.

Here, we aimed to advance current knowledge about the role of APP in inflammation and cognition. We tested the hypothesis that loss of APP function in mice would lead to cognitive loss and anxiety behavior, both of which are typically present in AD, as well as changes in the expression of inflammatory mediators. We report that App^{KO} mice displayed increased thigmotactic behavior compared to wildtype (WT) controls while retaining short-term spatial memory and long-term recognition memory. Systematic analysis of a broad spectrum of cytokines/chemokines associated with anxiety and cognitive loss further revealed that IL-13 and IP-10 expression in the brain was increased in App^{KO} mice, in line with current evidence supporting a role for these cytokines in anxiety behavior, both in rodents and humans.

2. Materials and methods

2.1 Animals

Animal study was approved by Loma Linda University Institutional Animal Care and Use Committee (LLU#8180006). Male and female wildtype (WT) C57BL/6J mice between ages of 12-18 weeks were purchased from Jackson Laboratory. Male and female App^{KO} (B6.129S7-App^{tm1Dbo}/J) mice were bought from Jackson Laboratory and bred in house. Male and female App^{KO} mice used for this study were 12–18 weeks old. Mice were kept on a 12-h light/12-h dark cycle, from 7 am to 7 pm, and with *ad libitum* access to food

(5LG4; LabDiet, Catalog #1818254-203) and water. Following behavioral and memory tests, mice were anesthetized with isoflurane and euthanized by transcardial puncture and blood collection, followed by decapitation.

2.2 Behavioral tests

Open field, novel object recognition, and Y-maze tests were used to assess behavior and cognition. Open field and novel object recognition tests overlapped, such that the open field test coincided with day 1 of habituation for novel object recognition test. There was a 3-day break between novel object recognition and Y-maze tests to allow for rest between both tests. Fig. 1 illustrates the timeline of the experimental design. Ethovision® XT was used to track and score the behavioral outcomes of the mice (Noldus, RRID: SCR_000441, RRID: SCR_004074). In all three tests, once the mouse was done with its task, the apparatus was cleaned with 70% ethanol, followed by distilled water to eliminate odor cues [12]. The raw output of the statistical analysis of all behavioral tests is provided in Supplemental Fig. 1.

The Open field test was used to measure emotionality [12, 13]. The mice were acclimated to the test room for at least 30 min before testing. Mice were placed in the center of the open field box (38 x 38 x 64 cm high) and recorded with Ethovision® XT for 10 min. At the end of the test, mice were placed in a separate holding cage. Once all mice were tested, they were returned to their home cages. Emotionality parameters like thigmotaxis, distance traveled, velocity and number of fecal droppings were measured using Ethovision® XT software.

The novel object recognition test relies on the natural curiosity and exploratory nature of mice and does not require excessive training [14, 15]. Mice were acclimated to the room for at least 30 min before testing. The test was performed across five days with a 24-hour inter-trial interval between the familiarization and testing phase to assess long-term recognition memory. The novel object recognition apparatus is quadrangular with dimensions of (38 x 38 x 64 cm high). The spatial cues in the room were minimized to promote the usage of hippocampal-independent pathways [16, 17]. During days 1–3, mice were habituated to the novel object recognition apparatus; day 4 was the familiarization phase, during which mice must reach 38 seconds of object exploration for each object within an allotted time of 15 min [18]. When a mouse reached 38 seconds of exploration for each object before 15 min, that would mark the end of the familiarization phase, and the mouse would immediately be removed from the testing arena. Day 5 was the testing phase, so after the inter-trial interval of 24 hours, the mice were placed in the room again, and one of the familiar objects was switched with a novel object. The mice were then given 5 min to explore. At the end of their test, mice were placed in a holding cage until tests were carried out for all mice. Then all mice were returned to their home cages. Long-term recognition memory was assessed by calculating the discrimination ratio. The formula used for the discrimination ratio is as follows:

$$discrimination\ ratio = \frac{Time\ spent\ exploring\ novel\ object}{Total\ time\ exploring\ both\ objects}$$

The Y-maze test also relies on the natural exploratory nature of mice [19, 20]. Mice were acclimated to the room for at least 30 min before testing. The Y-maze apparatus is made of transparent plexiglass walls to ensure visibility of spatial cues placed around the room. The Y-maze has three arms with dimensions of 8 cm x 30 cm x 8 cm (width, length, and height) and a 120° angle between each arm. Briefly, the test is conducted in two trials. First, the arms were designated as the start, novel, and other. The start arm is where the mouse is placed at the beginning of the trial. The novel arm is always blocked during trial 1, but unblocked during trial 2, and the other arm is the arm that is free for exploration during trials 1 and 2. The start, novel, and other arm were randomized between mice to control for arm-bias effects. During trial 1, the novel arm was blocked by an opaque guillotine door, and thus, the mouse was able to explore the start and other arm for 15 min. At the end of 15 min, the mouse was placed in a holding cage. The inter-trial interval time was 2 hours to assess short-term spatial memory [14, 21]. Before placing the mouse back in the maze, the guillotine door blocking the novel arm was removed. The mouse was then given 5 min to explore all three arms. At the end of the trial, the mouse was placed in a holding cage. Once testing was completed, all mice were placed in their home cages. Short-term spatial memory was assessed by calculating the percentage of time spent in the novel arm and the total percentage of the number of entries into each arm.

2.3 Tissue collection

Mice were euthanized by decapitation. The brain was immediately dissected and halved sagittally. One hemisphere was preserved first in 4% paraformaldehyde for 4 days and then put into a 30% sucrose in PBS solution for 4 days. Brain hemispheres were then submerged in cryomolds (Tissue-Tek Intermediate Cryomold 25608-924) containing O.C.T. compound (Fisher Scientific 23-730-571) and quickly frozen by placing on dry ice. Samples were then stored at −80°C until ready for cryosectioning. The other hemisphere was flash-frozen in liquid nitrogen and stored at −80°C.

2.4 Cytokine/chemokine detection

Cerebral cortex tissue from male and female mice were used to determine protein levels of 32 cytokines/chemokines (WT mice n=4 and App^{KO} mice n=4). Samples were thawed on ice, weighed, and homogenized in protein extraction buffer (Sterile PBS, 0.05% Triton X, HaltTM Protease Inhibitor Cocktail (Thermo Fisher Scientific, Waltham, MA) using 1.4 mm zirconium beads and benchtop BeadBug tissue homogenizer (Benchmark Scientific, Sayreville, NJ). Homogenates were sonicated for one min in a sonication bath (Branson M1800, Branson Ultrasonics, Danbury, CT) and centrifuged at 12,000g for 20 min at 4°C, as previously described [22-24]. A multiplexed magnetic bead-based immunoassay kit (Catalog# MCYTMAG-70K-P X 32, Millipore Sigma, Burlington, MA) was used to determine the levels of 32 cytokines/chemokines, according to the manufacturer's instructions. All data for cytokine/chemokine analysis were adjusted for brain wet weight and are represented as the mean ± standard error. Statistical significance between the two groups was evaluated by using the Student's t-test, with p-values < 0.05 considered statistically significant. The 32 molecules chosen for analysis, together with the rationale for their use, are presented in Supplementary Table 1 and listed here: eotaxin/CCL11, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12/p40,

IL-12/p70, IL-13, IL-15, IL-17, IP-10/CXCL10, KC/CXCL1, L.I.F., LIX/CXCL5, MCP-1/CCL2, M-CSF, MIG/CXCL9, MIP-1α/CCL3, MIP-1β/CCL4, MIP-2/CXCL2, RANTES/CCL5, TNF-α, and VEGF.

2.5 Immunohistochemistry

Brain tissue was cryosectioned coronally through the cerebral cortex at 20 μm/section on gelatin-chrome alum-coated Superfrost microscope slides (V.W.R., Denver, U.S.A.). Slides were placed on a warmer at 37 °C for 30 min and rinsed with PBS for 10 min six times. Slides were then incubated in blocking solution (PBS with 5% normal goat serum, 1% bovine serum albumin, and 0.2% of 10% Triton X-100) for 2 h at room temperature. This step was followed by a 4 °C overnight incubation with either IP-10 (1:50; Invitrogen 701225), IL-13 (1:100; Abcam ab106732), NeuN (1:2000; Abcam ab134014), IBA1 (1:200; Novus NB100-1028), or GFAP (1:2000; Novus NBP1-05198), antibodies; incubation buffer consisted of PBS with 2% normal goat serum, 1% bovine serum albumin, and 0.1% of 10% Triton X-100. Following 10 min washes 4 times in PBS with 0.1% Tween-20, slides were incubated in the dark with Alexa Fluor Plus 594 (goat anti-chicken; ThermoFisher Scientific A32759), Alexa Fluor 488 (donkey anti-rabbit; ThermoFisher Scientific A21206), Alexa Fluor Plus 594 (donkey anti-goat; ThermoFisher Scientific A32758) for 2 h at room temperature; incubation buffer consisted of PBS with 2% normal goat serum, 1% bovine serum albumin and 0.1% of 10% Triton X-100. Slides were washed for 10 min 4 times in PBS with 0.1% Tween-20 and twice (10 min each) with PBS. Slides were then treated with TrueBlack ® (Biotium 23007) for 30 seconds to minimize tissue autofluorescence. Slides were then washed with PBS for 10 min 3 times. Slides were mounted in Vectashield/DAPI hard-set mounting medium (Vectashield H-1500). Three mice per group (n=3) were used for all immunohistochemistry experiments.

3. Results

3.1 *App*^{KO} mice demonstrated increased thigmotactic behavior

The open field test was used to determine thigmotaxis, which is the tendency for a mouse to seek out the borders versus the center when placed in an open area. Mice that remain close to the walls longer are classified as more anxious than mice that venture out to the center [13]. In our hands, *App*^{KO} mice showed decreased time in the center ($p=0.0062$ between males and $p<0.0001$ between females) and increased time spent in the borders compared to WT mice ($p=0.0308$ between males and $p=0.0128$ between females) (Fig. 2A, C). Together these results indicate increased thigmotactic behavior in the *App*^{KO} mice. There were no significant differences in total distance traveled between groups ($p=0.9961$ between males and $p=0.8954$ between females) (Fig. 2B). Other emotionality parameters like velocity and fecal counts were measured but there were no statistically significant differences between groups (Supplemental Fig. 2).

3.2 Neither spatial nor long-term recognition memory were impaired in *App*^{KO} mice

The Y-maze test was used to assess hippocampal-dependent short-term spatial memory. Before testing, objects were placed around the maze to serve as spatial references for the mice and ensure that spatial memory was tested [19]. Fig. 3A shows no significant

difference between groups in the percent of time spent in the novel arm (p=0.8994 between males, p=0.1000 between females). Also, both WT and *App*^{KO} mice preferred the novel arm (Fig. 3B).

We then carried out a version of the novel object recognition test in which spatial cues are minimized to promote engagement of hippocampal-independent memory pathways [16, 17]. Fig. 3C shows there was no difference between groups in long-term recognition memory (p=0.9991 between males, p=0.3056 between females), as determined by the discrimination ratio. Note also that scoring above a ratio of 0.5 is indicative of novel object preference.

3.3 *App*^{KO} mice had increased levels of IP-10 and IL-13 in the brain cortex

Our results confirmed that loss of APP affects thigmotaxis, but both short-term spatial and long-term recognition memory remains intact. We then asked whether loss of APP could also lead to changes in inflammatory status in the brain. To answer this question, we carried out a multiplexed magnetic bead-based immunoassay designed to detect 32 cytokines/chemokines linked to AD and anxiety (Supplemental Table 1). Of all the candidate markers tested, IP-10 and IL-13 levels were increased in *App*^{KO} mice compared to WT mice, p=0.0250 and p=0.0002, respectively (Fig. 4). IL-1α, eotaxin, IFN-γ, IL-10, IL-15, IL-6, IL-7, IL-9, K.C., MCP-1, M-CSF, M.I.G. and MIP-1α were detected but statistical analyses revealed no significant differences between groups (Fig. 5).

4. Discussion

This study aimed to test the hypothesis that loss of APP function in mice may lead to changes in the expression of inflammatory mediators known to be associated with cognitive impairment and anxiety-related behavior. Our findings showed that *App*^{KO} mice displayed increased thigmotaxis, as measured by the open field test, relative to WT mice (Fig. 2A, C). Short-term spatial memory, as measured by the Y-maze test (Fig. 3A), and long-term recognition memory as measured by the novel object recognition test, were not impaired in *App*^{KO} mice (Fig. 3C). Multiplexed immunoassay analysis of the cerebral cortex revealed a significant increase in levels of IP-10 and IL-13 (Fig. 4), but no changes in the levels of other inflammatory mediators (Fig. 5).

Our behavioral findings possibly reflect a specific impact on hippocampal function induced by the loss of APP. The hippocampus can be functionally divided into dorsal and ventral regions. While the dorsal hippocampus is associated with memory function, including spatial memory, the ventral hippocampus is involved in regulating emotional behavior, including anxiety and fear [25, 26]. In addition, lesions to the ventral hippocampus in rodents affect anxiety-related behaviors [27, 28].

The increased thigmotaxis that we report in our *App*^{KO} mice (Fig. 2) is in line with similar changes in thigmotactic behavior reported by other labs [29, 30]. Thigmotaxis is commonly used to measure anxiety, suggesting that the increase in thigmotactic behavior seen in *App*^{KO} mice (Fig. 2) could reflect increased anxiety-like behavior in these mice [13]. Analysis of other emotionality parameters, namely velocity in the open field test, and fecal dropping counts (Supplemental Fig. 2), showed no differences between groups. Similar

uncoupling between thigmotaxis and other parameters of emotionality has been previously reported [31], although the implications of such outcomes in terms of behavioral evaluation are unclear. Figure 2 also showed no differences in total distance traveled between WT and App^{KO} mice. This is an apparent contrast to the original report from the Zheng lab describing diminished locomotor activity in the App^{KO} mice. However, this lab used an actophotometer to measure beam breaks to evaluate locomotor activity but did not measure total distance traveled. Therefore, we do not know whether their mice and our mice walked the same distance and have similar locomotion [9].

In contrast, short-term spatial memory in App^{KO} mice was not impaired as measured by Y-maze (Fig. 3A, B). Interestingly, rats with the APP Swedish mutation ($APP_{Swedish}$) also showed increased anxiety but intact spatial memory, suggesting that both functional inactivation of APP (as present in our App^{KO} mice) and aberrant gain of function (as present in $APP_{Swedish}$ rats) may share at least some of the mechanisms of disease pathogenesis [32]. This notion is further supported by our previous work reporting that the brains of both App^{KO} mice and sporadic AD patients show a defective protective mechanism against 27-hydroxycholesterol, a cholesterol metabolite that accumulates in the sporadic AD brain [33]. The novel object recognition test evaluated long-term recognition memory, which is dependent on the perirhinal cortex but not on hippocampal function [34]. Long-term recognition memory was unaffected in App^{KO} mice based on our findings in Fig. 3C.

It is also important to note that our behavioral analyses were performed with 12–18-week-old mice; it is possible that, as the mice develop more severe inflammation dysregulation with age, behavioral differences could widen. Future studies will determine whether the differences we see in our mice are age dependent.

Next, to evaluate inflammatory changes associated with APP loss, we quantified cytokines and chemokines previously linked to cognitive and anxiety impairment. The complete list of these markers, and their phenotypic association, is presented in Supplemental Table 1. Our multiplexed immunoassay data showed significantly increased expression of IP-10 and IL-13 in App^{KO} mice compared to WT mice (Fig. 4). IP-10 is a cytokine important for leukocyte migration but its role in the brain is unclear. Notably, in a lipopolysaccharide-induced anxiety/inflammation mouse model, mice treated with lipopolysaccharide displayed a significant increase in IP-10 expression in the brain, which correlated with increased anxiety [35].

Much of our knowledge on the cytokine IL-13 is based on its role in the peripheral immune system; it is produced primarily by T-helper type 2 lymphocytes and is typically considered an anti-inflammatory cytokine that downregulates the synthesis of T-helper type 1 pro-inflammatory cytokines [36]. In the brain, it is expressed by microglia in rats and by neurons in gerbils [37, 38]. Functionally, IL-13 can be both neuroprotective by reducing inflammation but also neurotoxic by increasing the susceptibility of neurons to oxidative damage [36]. Because loss of APP decreases mitochondrial function and increases oxidative stress in multiple cell types, including cortical neurons [39, 40], it is possible that increased IL-13 expression in App^{KO} mice increases the susceptibility of neurons to oxidative damage and compromises neuronal circuitry in areas associated with anxiety. Interestingly, IL-13 has

been implicated in two different anxiety models, one induced by allergic rhinitis and the other by lipopolysaccharide [41, 42]. Furthermore, in pregnant women, IL-13 was one of the cytokines positively correlated with increased anxiety [43]. Overall, these findings strongly suggest a role for IL-13 in regulating anxiety behavior, and its regulation could be the basis for therapeutic approaches to help reduce anxiety.

In a previous publication we assessed gliosis in App^{KO} mice and found no astrogliosis but minor microgliosis [7]. Interestingly, Zheng et al reported astrogliosis in only 66% of the App^{KO} mice. To us, this outcome strongly suggests an inherent biological variability in glial activation in these mice and supports the notion that APP loss leads to changes in the expression of select cytokines in the absence of gliosis-driven broad cytokine production [9].

Our results provide a basis for future exploration of expression of IL-13 and IP-10 in brain regions involved in anxiety neurocircuitry and cell source of these cytokines. In that regard, qualitative immunohistochemistry shows that App^{KO} mice display an apparently larger number of cells positive for IL-13 in the medial prefrontal cortex and ventral hippocampus, regions implicated in emotional regulation and anxiety [44, 45] (Supplemental Figs. 3-5). We have also identified neurons as the sole source of IP-10 in the medial prefrontal cortex and hippocampus in our mice, at least as measured by the presence of the marker NeuN (Supplemental Figs. 6-8). Future work will fully characterize the cellular sources and brain regions involved in the changes observed in our multiplexed analysis of IP-10 and IL-13 levels in App^{KO} mice (Fig. 4).

In summary, our findings demonstrate that loss of APP function in mice increased thigmotaxis as measured by the open field test but has no significant effects on short-term spatial memory or long-term recognition memory. Also, App^{KO} mice showed increased brain expression of IP-10 and IL-13, both of which have potential roles in inducing anxiety behavior. Future studies will determine the potential of optimizing IL-13 and IP-10 signaling as a viable effective therapy for reducing anxiety, and whether dysregulation of these inflammatory mediators contributes to AD pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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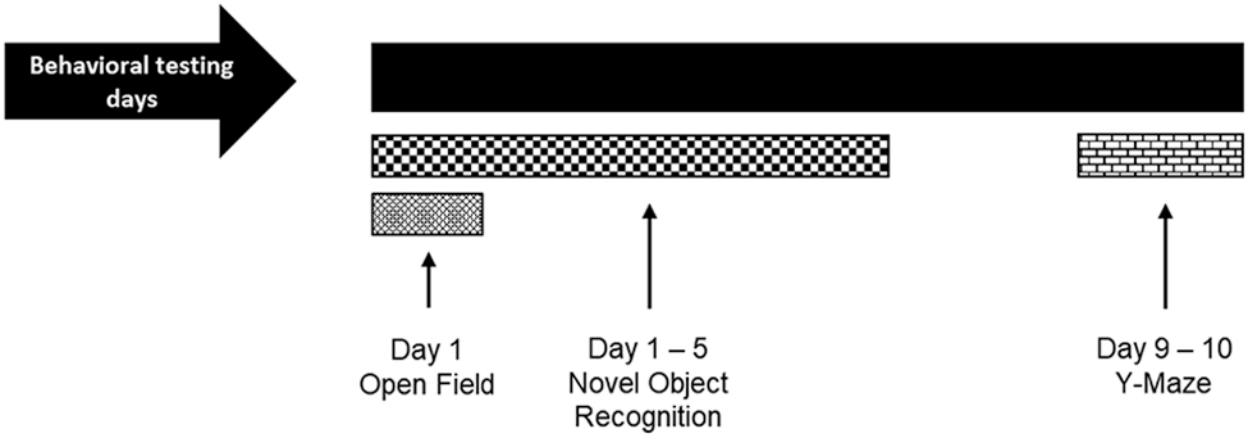

Fig. 1.

Illustration of the order of behavioral tests for WT and App^{KO} mice. A full explanation is described in the main text.

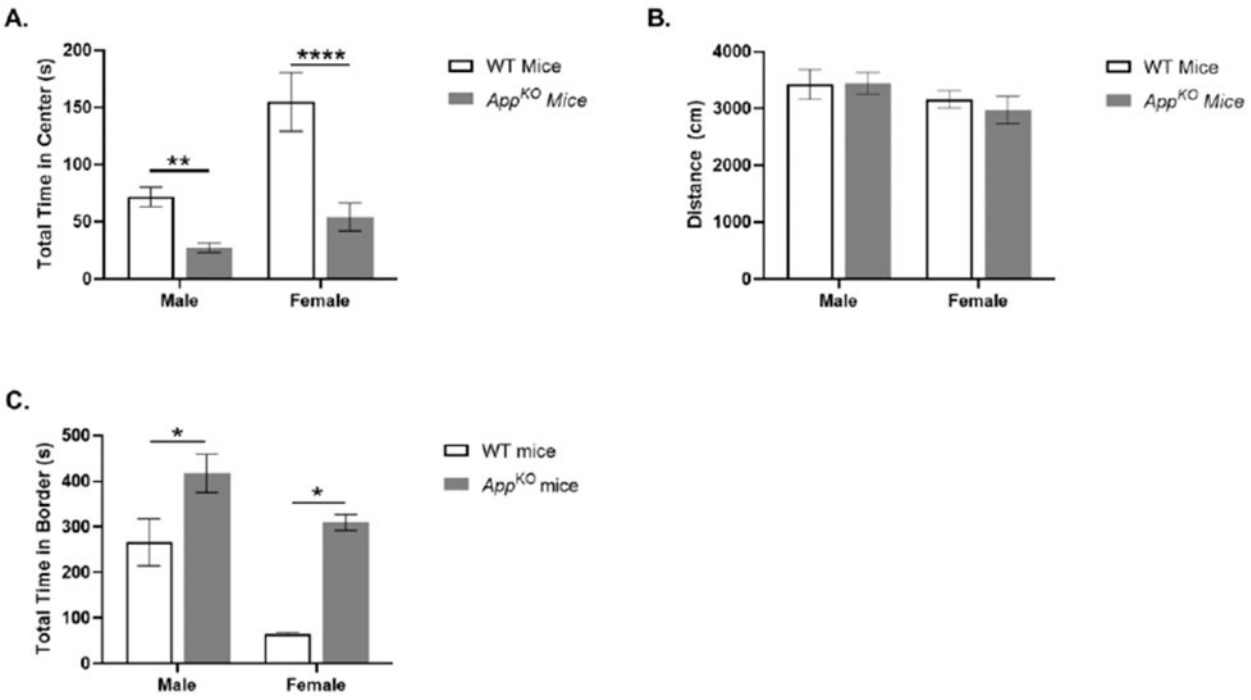

Fig. 2.

Male and female *App*^{KO} mice demonstrated thigmotactic behavior. After mice were placed in the open field box for 10 min, time in the center and borders were calculated. **A)** *App*^{KO} male and female mice displayed decreased time spent in the center compared to WT male and female mice ($p=0.0062$ for males and $p<0.0001$ for females). **B)** There was no significance in total distance traveled between WT and *App*^{KO} males ($p=0.9961$) or WT and *App*^{KO} females ($p=0.8954$). **C)** *App*^{KO} male and female mice displayed increased time spent in the borders compared to WT male and female mice ($p=0.0308$ for males and $p=0.0128$ for females). WT male mice n=10, *App*^{KO} male mice n=8, WT female mice n=4 and *App*^{KO} female mice n=5. Data expressed as ± S.E.M. *$p<0.05$ vs. WT mice, **$p<0.01$ vs. WT male mice ****$p<0.0001$ vs. WT female mice, two-way ANOVA.

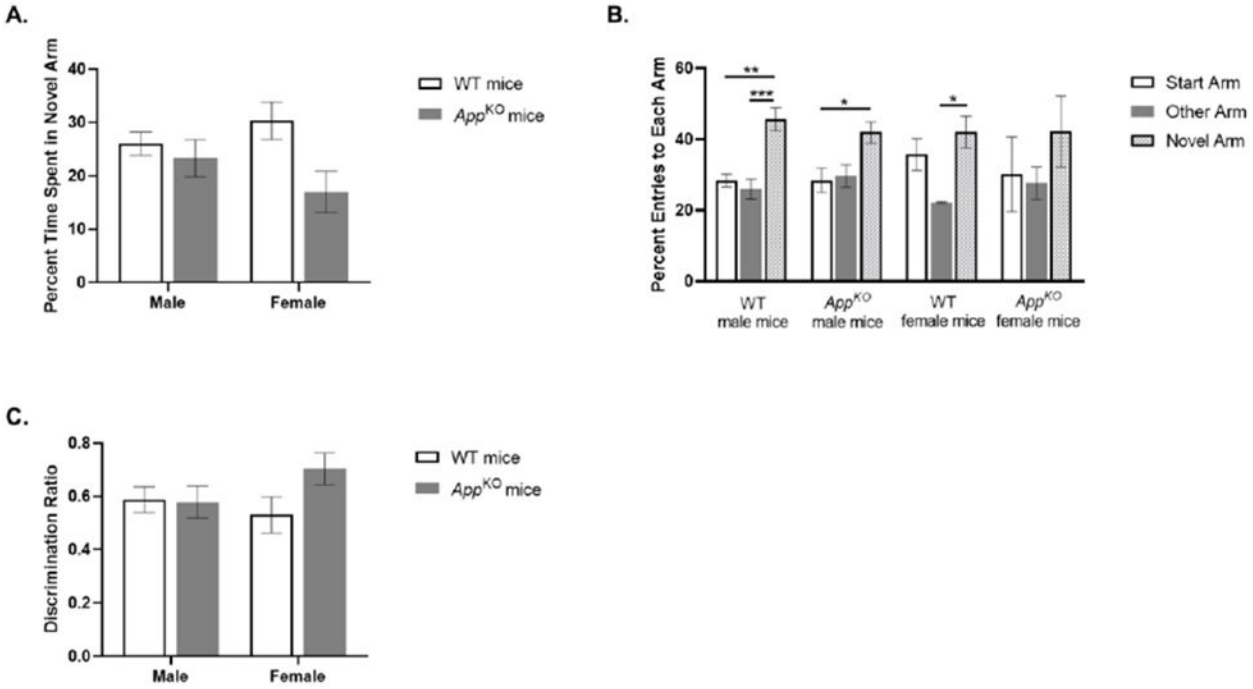

Fig. 3.

Loss of APP in male and female mice did not affect short-term spatial memory nor long-term recognition memory. **A)** There was no significant difference in percent time spent in the Y-maze novel arm between the genotype groups (p=0.8994 between males, p=0.1000 between females). **B)** Percent of visits to each arm also showed that both WT and *App*^{KO} mice preferred the novel arm. WT male mice preferred the novel arm versus start arm (p=0.0030) and other arm (p=0.0006). *App*^{KO} male mice preferred the novel arm versus start arm (p=0.0491). WT female mice preferred the novel arm versus other arm (p=0.0389). **C)** There was no significant difference between groups in long-term recognition memory (p=0.9991 between males, p=0.3056 between females). **A, B)** Y-maze test, WT male mice n=10, *App*^{KO} male mice n=8, WT female mice n=4, *App*^{KO} female mice n=5. **C)** Novel object recognition test, WT male mice n=10, *App*^{KO} male mice n=6, WT female mice n=4, *App*^{KO} female mice n=5. Data expressed as ± S.E.M., *p<0.05 vs. novel arm, **p<0.01 vs. novel arm, ***p<0.001 vs. novel arm, Two-way ANOVA.

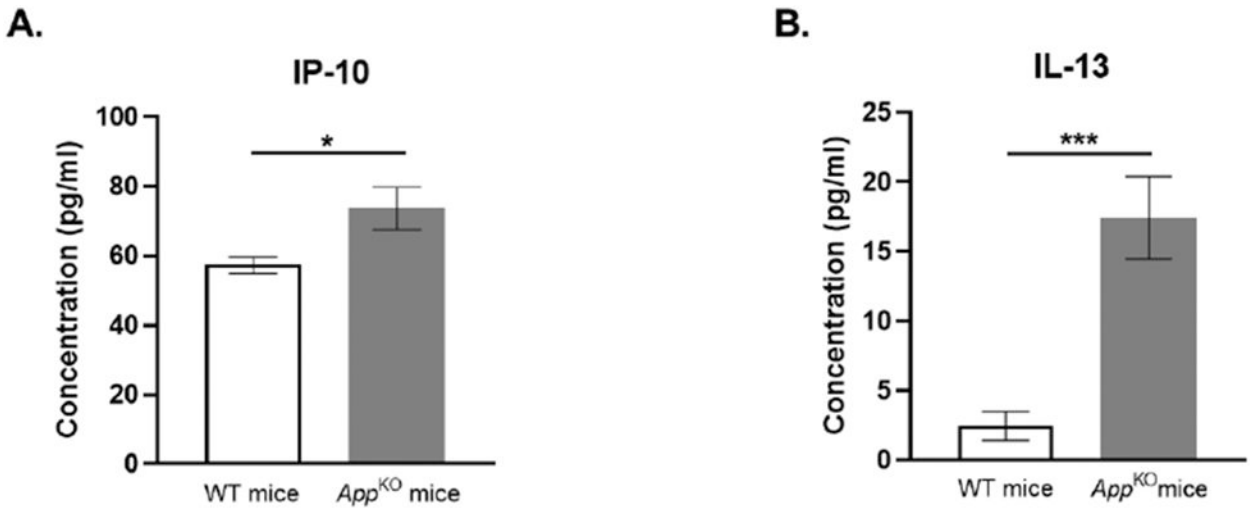

Fig. 4.

Cytokine/chemokine expression in the cerebral cortex of WT and *App*^{KO} mice. **A)** *App*^{KO} mice had increased IP-10 levels (p=0.0250). **B)** *App*^{KO} mice had increased IL-13 levels (p=0.0002). WT mice n=4 and *App*^{KO} mice n=4. Data expressed as ± S.E.M. *p<0.05 vs. WT mice, ***p<0.001 vs. WT mice; unpaired *t*-test.

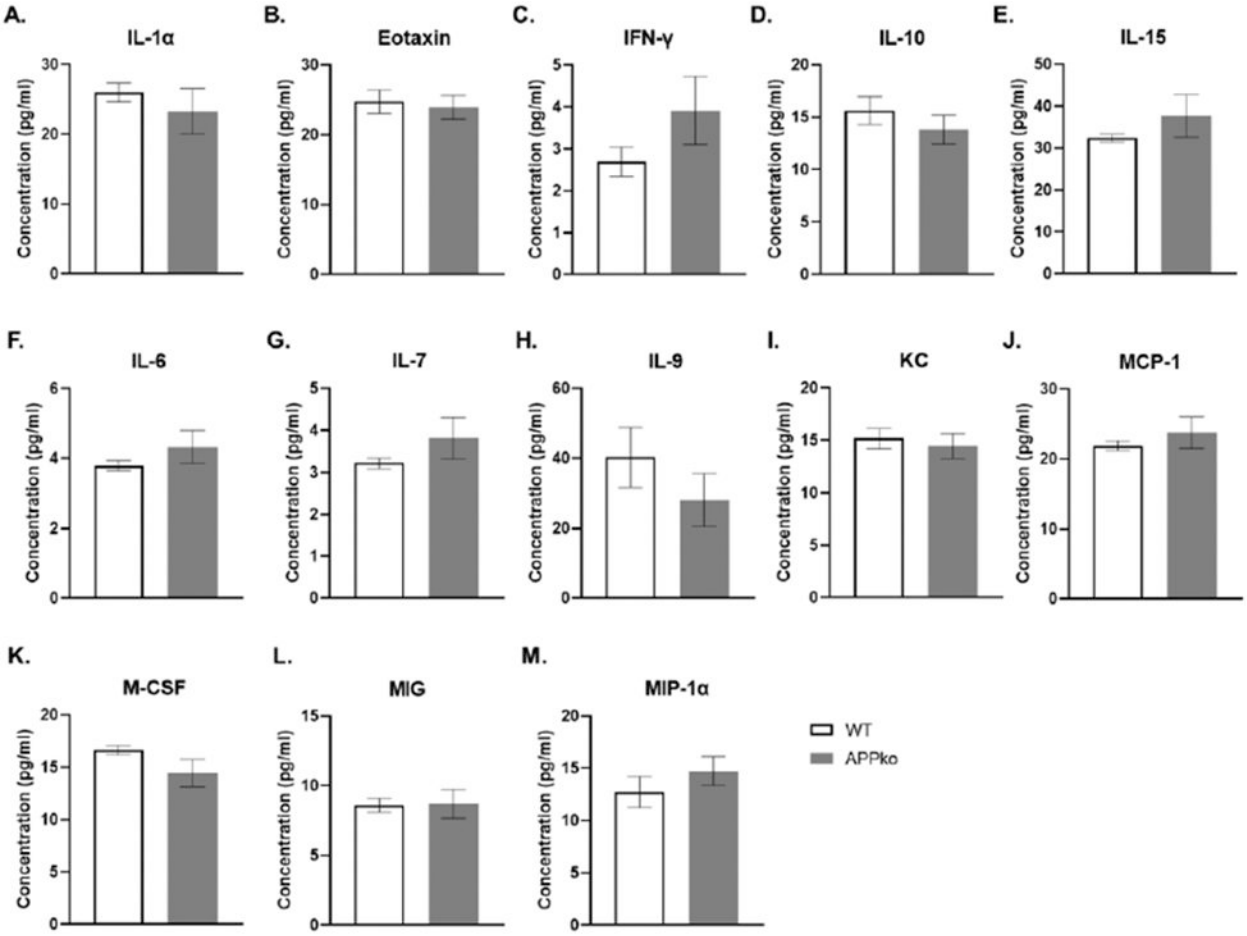

Fig. 5.

Thirteen cytokines/chemokines did not show significant differences between WT and *App*^{KO} mice. WT mice n=4 and *App*^{KO} mice n=4. Data expressed as ± S.E.M., unpaired t-test.