IL-17A Contributes to the Angiotensin II-induced Neurovascular Coupling impairment through Oxidative Stress

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ABSTRACT

Hypertension, a multifactorial chronic inflammatory condition, is a risk factor for neurodegenerative diseases including stroke and Alzheimer's disease. These diseases have been associated with higher concentration of blood interleukin (IL)-17A. However, the role that IL-17A plays in the relationship between hypertension and brain remains misunderstood. Cerebral blood flow regulation may be the crossroads of these conditions. Hypertension alters cerebral blood flow regulation including neurovascular coupling (NVC). In the present study, the effects of IL-17A on NVC in the context of hypertension induced by angiotensin (Ang) II will be examined. Our results show that the neutralization of IL-17A or the specific inhibition of its receptor prevent the Ang II- induced NVC impairment. These treatments reduce the Ang II- induced cerebral oxidative stress. Tempol and NOX-2 depletion prevent NVC impairment induced by IL-17A. These findings suggest that IL-17A, through superoxide anion production, is an important mediator of cerebrovascular dysregulation induced by Ang II.

INTRODUCTION

Hypertension, now considered a chronic inflammatory condition, is the most prevalent and modifiable risk factor for neurodegenerative diseases including stroke and Alzheimer's disease¹. However, although many studies have linked brain inflammation and cerebrovascular alterations with brain disorders²⁻⁵, the involvement of inflammation in the impact of hypertension on the brain remains largely unknown. Previous data from experimental model of hypertension have

shown important alteration of cerebral blood vessels and cerebral blood flow (CBF) regulation including neurovascular coupling (NVC) ⁶⁻⁸. NVC is defined by an increase in CBF in response to neuronal activation. Since the brain has high energy needs, slight alterations of this mechanism can negatively impact cerebral protein synthesis and neuronal function⁹. Although the involvement of inflammation in hypertension and peripheral vascular injury is well documented, the impact on CBF regulation remains largely underinvestigated.

The impact of inflammation on NVC was previously revealed through anti-inflammatory treatments with Treg lymphocytes (CD4+/CD25+) or interleukin (IL)-10 in an experimental model of hypertension induced by mice chronic angiotensin (Ang) II perfusion. In this study, we have shown that these treatments prevent gliosis and cerebral oxidative stress⁶. Ang II plays a key role in inflammation and increases the activity of T effector cells such as T helper 17 lymphocytes and gamma-delta T-cells,^{10,11} enhancing the production of pro-inflammatory cytokines, such as IL-17A^{10,12}. IL-17A can induce brain damage by acting directly on neurons¹³ or indirectly through disruption of the BBB and neurovascular functions ¹⁴⁻¹⁷. We hypothesized that these effects could be achieved through oxidative stress, since nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, specifically NOX-2, derived reactive oxygen species are well known to promote cerebrovascular dysfunctions¹⁸⁻²⁰ and to be involved in IL-17A-induced vascular inflammation²¹.

IL-17A is involved in reduced resting CBF and NVC impairment induced by high salt diet in mice¹⁶. These findings raise the possibility that IL-17A may be involved in CBF impairment in other experimental models of hypertension. Therefore, we examined the role of IL-17A in the

neurovascular dysfunctions induced by Ang II. To explore this question, we have characterized the impact of IL-17A on CBF regulation in Ang II-induced hypertensive mice. Then, the potential role of NOX-2 and the subsequent superoxide anion production were evaluated to explain how IL-17A impairs NVC.

RESULTS

Neutralization of IL-17A or inhibition of its receptor prevents the Ang II-induced NVC impairment

To examine the involvement of IL-17A on neurovascular coupling (NVC) impairment induced by Ang II, IL-17A neutralizing antibody (Ab) was administered in mice in parallel with Ang II. As previously observed⁸, CBF increases in response to whiskers stimulations in Ang II hypertensive mice were significantly attenuated by 23.2% compared to their controls (Figure 1 A, C, $p < 0.01$). Neutralizing IL-17A Ab prevents NVC impairment induced by Ang II (Figure 1 A, D, $p < 0.01$) without changes in resting CBF (Figure 1B). IL-17A Ab does not elicit changes in cerebrovascular responses to neuronal stimulations in control mice.

We next examined whether the rescued cerebrovascular responses corresponded to a prevented increase in systolic blood pressure (SBP). In mice not receiving the IL-17A Ab, as expected^{10,22,23}, Ang II significantly increased the SBP compared to its control at day 7 and 14. This increase in SBP is of 37.4 and 35.9 mmHg respectively. IL-17A neutralization slightly attenuates the increase in SBP induced by Ang II at those same time point by 9.8 and 9.4 mmHg (Supplemental Figure 2).

In order to assess whether the IL-17A prevents NVC impairment through its receptor, mice received an IL-17A receptor antagonist (IL-17RA mAB) simultaneously with the chronic systemic administration of Ang II. Ang II attenuates the increases in CBF (13.6%) in response to whiskers stimulations compared to the control sham mice (18.3%) (Figure 2 A, C, $p < 0.01$). Inhibiting the IL-17A receptor prevents the disruption of NVC induced by Ang II (18.6%) (Figure 2 A, D, $p < 0.01$). The laser-Doppler probe was positioned to get similar resting CBF in between groups (Figure 2B). The receptor antagonist does not elicit changes in cerebrovascular responses in control mice.

Then, the effect of IL-17A receptor inhibition on the SBP increase induced by Ang II was determined. The mean SBP in mice receiving Ang II was significantly higher compared with controls at day 7 and 14 with an increase of 51.7 and 53.7 mmHg, respectively. Inhibition of the IL-17A receptor partially attenuated the increase in SBP induced by Ang II at those same day point by 11.4 and 10.1 mmHg. (Supplemental Figure 3).

Neutralization of IL-17A or inhibition of its receptor prevents the superoxide anion production induced by Ang II

To determine whether the increase production of reactive oxygen species by Ang II is mediated by IL-17A, we investigated the effect of IL-17A Ab on superoxide anion production. As shown in Figure 3, the increased production of superoxide anion by Ang II seen in the somatosensory cortex ($p = 0.12$) and in the lacunosum moleculare (LMol), dentate gyrus (DG), cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3) regions of the hippocampus ($p < 0.01$) was prevented

following IL-17A Ab administration ($p < 0.05$). Moreover, the production of superoxide anion in hypertensive mice receiving IL-17A Ab does not differ from that of control mice (Sham) with or without the IL-17A Ab administration.

In the same manner, somatosensory cortex and in all regions in hippocampus (LMol, DG, CA1, CA3,) showed a higher production of superoxide anion in mice with chronic administration of Ang II. IL-17RA mAB administration normalized these levels to the same as those of control mice (Sham) with or without the IL-17RA antagonist (Figure 4).

Tempol treatment or NOX-2 depletion prevents NVC dysfunction induced by IL-17A

Since IL-17A neutralization and IL-17A receptor inhibition prevent NVC impairment and oxidative stress induced by chronic systemic administration of IL-17A, we tested whether superoxide anions mediate the effects of IL-17A on NVC. Mice were thus chronically treated with the antioxidant superoxide scavenger and superoxide dismutase (SOD)-mimetic Tempol. As shown in figure 5 A and C, systemic IL-17A Rb administration reduced CBF increase in response to whiskers stimulations by 22.8% compared to controls ($p < 0.01$). Disruption of NVC by the IL-17A Rb is prevented by Tempol. CBF responses were not modulated by Tempol alone (Figure 5 A, D, $p < 0.05$). The resting CBF was similar in all groups (Figure 5B).

To better understand the mechanisms by which IL-17A induces cerebral oxidative stress, we investigated the implication of NOX-2, the isoform present in brain endothelial cells, microglia and astrocyte^{7,19,24}, on NVC by using knock out mice (NOX-2^{-/-}). As obtain previously, IL-17A Rb administration in WT mice attenuated the response to whiskers stimulations by 24.9%

compared to control (Figure 6 A, C, $p < 0.05$). Interestingly, NOX-2^{-/-} mice do not exhibit NVC impairment or change in resting CBF in response to IL-17A Rb administration (Figure 6 A, B, D, $p < 0.05$). We next examined whether the rescue in cerebrovascular responses correspond to a decrease in systolic blood pressure (SBP) in NOX-2^{-/-} mice. Results from Supplemental Figure 4B, shows that the deletion of the NOX-2 gene does not prevent the increase in SBP observed at day 7 in response to IL-17A Rb administration (151.4 mmHg in WT vs 152.0 mmHg NOX-2^{-/-} mice).

Tempol treatment or NOX-2 depletion prevents superoxide anion production induced by IL-17A

We then tested the efficiency of Tempol and NOX-2 to normalize the superoxide anion production induced by IL-17A. Figure 7 shows a significantly higher production of superoxide anion in somatosensory cortex and all hippocampal regions (LMol, DG, CA1, CA3) ($p < 0.05$) in mice receiving the IL-17A Rb. In those same regions, Tempol prevents these increases without modulating the resting superoxide levels. Similarly, NOX-2^{-/-} mice that received IL-17A Rb presented similar level of superoxide anion production in the somatosensory cortex and all regions of the hippocampus as in control mice (Figure 8, $p < 0.05$).

DISCUSSION

Our major new findings are that IL-17A, through its receptor, IL-17RA, takes part in the NVC impairment induced by Ang II. This effect is obtained through a process mediated by the NOX-2-induced superoxide anion production in the hippocampus and the somatosensory cortex as demonstrated by a treatment with Tempol and deletion of the gene coding for NOX-2.

Ang II through the Ang II type 1 receptor (AT₁R) signaling pathway is an important proinflammatory stimulus, triggering the production of cerebral and systemic proinflammatory cytokines^{6,25-27}, chemokines²⁸ and reactive oxygen species^{7,10,19,29-31}. The presence of perivascular macrophages expressing AT₁ receptors in the neurovascular unit led to the hypothesis that inflammatory factors may mediate the effect of Ang II. Perivascular macrophages depletion indeed rescued NVC in Ang II slow pressor hypertension³². The modulatory impact of cytokines on NVC was then demonstrated in the Ang model of hypertension⁶. In this study, the anti-inflammatory cytokine, IL-10, also prevented gliosis and cerebral oxidative stress⁶. Inversely, a systemic inflammatory state in mice characterized by higher circulating IL-17A levels and induced by a high salt diet, contributed to NVC impairment¹⁶. In the present study, blocking IL-17A or its receptor prevented the NVC impairment observed in Ang II slow pressor hypertension. Overall, these results suggest an important contribution of IL-17A in NVC impairment in models of hypertension. However, the mechanism by which IL-17 impairs NVC remains to be established.

Previous reports have shown an important link between the effect of Ang II on peripheral endothelial function and IL-17A^{10,33} characterized by a lower increase in blood pressure in Ang II-induced hypertensive mice receiving IL-17A Ab or IL-17RA mAB administration³³ or in IL-17^{-/-} mice¹⁰. Our results confirmed the reduction in blood pressure in mice receiving these treatments. However, these changes in blood pressure cannot explain the impact on NVC since it was previously demonstrated that the impact of Ang II on NVC is independent of its hypertensive effect^{8,27}.

A more probable mechanism by which Ang II exerts its deleterious actions on NVC would be by activating its AT1R, at least on endothelial cells and perivascular macrophages^{7,32}, which subsequently lead to the production of reactive oxygen species^{7,19,24}. In the periphery, blood vessels from IL-17^{-/-} mice preserved vascular function, decreased superoxide production, and reduced T-cell infiltration in response to Ang II¹⁰. Therefore, we hypothesized that IL-17A could also modulate NVC in Ang II-induced hypertensive mice through a similar pathway. Our results confirm increased superoxide anion production in the hippocampus and somatosensory cortex in the slow pressor model induced by Ang II. Interestingly, neutralizing IL-17A or inhibiting its receptor normalizes the superoxide anion production. These results match those observed in the periphery by Madhur et al¹⁰. Overall, these findings suggest that IL-17A is involved in the superoxide anion production induced by Ang II, but that it is part of the mechanism by which Ang II impairs NVC.

To demonstrate that IL-17A can impair the cerebrovascular response, we tested the effect of the IL-17A Rb on NVC. We first showed that IL-17A Rb administration impaired NVC in a dose-dependent manner, where the 50 pg/kg/h dose showed a decrease in CBF in response to whiskers stimulations similar to the one seen in Ang II-induced hypertensive mice. However, it is not clear to what extent, the chosen concentration corresponds to physiopathological levels observed in humans. The IL-17 levels in plasma of hypertensive participants varie substantially with the duration of hypertension, the antihypertensive medication and comorbidities³⁴, and no study had established the link between IL-17 levels and end-organ damages. Thus, further studies with

large clinical cohorts will be necessary to establish the levels of IL-17 associated with cerebrovascular dysfunctions.

Similarly to Ang II in the slow pressor model of hypertension³⁵, IL-17A can cross the blood brain barrier and produce cerebral superoxide anion which can lead to cerebrovascular dysfunction¹⁵. Therefore, we investigated the role of oxidative stress in the IL-17A-induced NVC dysfunction. In this study, mice were treated with the antioxidant Tempol due to its ability to easily cross membranes and its stronger therapeutic effect compared with other frequently used antioxidants³⁶. Tempol treated mice show a CBF response to neuronal activation similar to those observed in control mice, showing the impact of oxidative stress on neurovascular decoupling induced by IL-17A. This has been confirmed by the preventive effect of Tempol on cerebral superoxide anion production in IL-17A Rb administered mice. These results suggest that increased superoxide anion production is a key mediator of IL-17A-induced NVC impairment.

NADPH oxidase, specifically NOX-2, the isoform expressed in cerebral endothelial cells, microglia and astrocytes, is likely the main source of cerebral superoxide anion in Ang II-induced hypertensive mice^{7,19,24}. In addition, NOX-2^{-/-} mice are protected from the Ang II on NVC⁷, further confirming the role of oxidative stress on cerebrovascular dysfunctions. We thus investigated the importance of NOX-2 on cerebrovascular dysfunction and oxidative stress in response to IL-17A. Our results confirm that NOX-2 plays role in NVC impairment induced by IL-17A, showing a similar CBF responses to whiskers stimulations and superoxide anion levels in NOX-2^{-/-} mice compared with their corresponding wildtype. This coincides with results observed in mice aortic vascular smooth cells where IL-17A induced reactive oxygen species

formation through NOX-2 activation²¹. However, even though a peripheral cardiovascular protection is possible, there was no difference in blood pressure between NOX-2^{-/-} mice and their wildtype. This supports previous results where NOX-2^{-/-} mice receiving similar doses of Ang II as in the present study (764ng/kg/min) do not present a lower systolic blood pressure³⁷.

In conclusion, we have demonstrated that IL-17A, through superoxide anion production, is an important modulator of NVC impairment induced by Ang II. Taken together, our findings suggest that modulating the immune system and targeting inflammation in hypertension could be a promising approach for reducing cerebrovascular dysfunctions. Given that hypertension and chronic inflammation are important risk factors for stroke, vascular cognitive impairment and Alzheimer's disease, the results of this study could open the door for future investigations to examine the influence of the immune system and inflammation on brain degeneration.

METHODS

This article adheres to the Transparency and Openness Promotion (TOP) Guidelines. The corresponding author can provide all data, methods, and materials used to conduct the research upon reasonable request.

Animals

The study was approved by the Committee on Ethics of Animal Experiments of Université de Montréal and performed in accordance with the guidelines of the Canadian Council for Animal Care and by the ARRIVE (Animal Research: Reporting of In Vivo Experiments). Ten- to twelve-weeks-old C57BL/6 male mice from Charles River Laboratories (Saint-Constant, Canada) were housed individually in a temperature-controlled room with ad libitum access to water and a

standard protein rodent diet (Tekland global 18% protein rodent diet). Ten-weeks-old C57BL/6 male mice with a targeted genetic deletion of NOX2 (B6.129S-Cybbtn1Din/J; Stock No: 002365) and their controls were obtained from Jackson Laboratory (Main, USA). Given that female mice are protected from the deleterious effects of Ang II on cerebrovascular functions³¹, only male mice were used in this study. Following acclimatization, animals were randomly assigned to experimental groups.

Drugs Administration

Osmotic minipumps (model 1002; Alzet, USA) containing Ang II (MilliporeSigma, USA) were implanted subcutaneously under isoflurane anesthesia as described previously³⁸. Briefly, mice received bupivacaine hydrochloride (Marcaine; CDMV, Canada, 2 mg/kg s.c.) at the site of the incision before the osmotic pump implantation. Each osmotic pump delivered 600 ng/kg/min of Ang II for 14 days. Control animals were sham-operated since no difference in cerebrovascular response was observed between mice with a sham surgery and those with an implantation of a saline-infused minipump in pilot experiments⁶. Starting on the day of the implantation, mice were injected intraperitoneally (i.p.) every four days with an IL-17A neutralizing antibody (eBioMM17F3; eBioscience, 0.5 µg/µL), a specific IL-17A receptor antagonist (PL-31280; Amgen, 0.5 µg/µL), or an immunoglobulin G (IgG) isotype control (0.5 µg/µL Invitrogen, Canada). This administration regimen was chosen based on prior studies on murine models of hypertension and atherosclerosis^{33,39,40}.

In another group of animals, systemic infusion of 50 pg/kg/h of mouse recombinant IL-17A (IL-17A Rb; 421-ML/CF; R&D system, USA) for 7 days was achieved via an osmotic minipump

(model 1007D; Alzet, USA). Since no study has previously shown the effect of systemic infusion of IL-17A on NVC, a dose-response curve of IL-17A Rb on cerebrovascular responses was assessed. IL-17A Rb administration has shown a dose-dependent effect on CBF in response to whiskers stimulations, where the 50 pg/kg/h dose showed a decrease in cerebrovascular response similar to the one seen in Ang II-induced hypertensive mice (Supplemental Figure 1). A subgroup of C57BL/6 mice were simultaneously treated with Tempol (4-hydroxy-TEMPO; Millipore Sigma, Canada; 1 mmol/l) administered in the drinking water or its vehicle (regular drinking water). Treatment with Tempol started 2 days before the osmotic pump implantation and ended at the time of sacrifice (one week after surgery).

Systolic blood pressure monitoring

Systolic blood pressure was monitored in awake mice using tail-cuff plethysmography (Kent Scientific Corp, Torrington, CT). Mice were warmed on a heating pad preheated at 37 °C for ten minutes before and during blood pressure recordings. Animals were habituated to the procedure three days before blood pressure assessment. Right before the implantation of osmotic minipump (day 0) and weekly until the NVC analysis, ten blood pressure assessment per mice were done and average. Blood pressure was monitored by the same person at the same time of the day.

Neurovascular coupling

Anesthesia was initiated with isoflurane (induction: 5%, maintenance: 2%) and maintained with 50 mg/kg of α -chloralose i.p. (MilliporeSigma, USA) and 750 mg/kg of urethane i.p. (MilliporeSigma, USA). The depth of anesthesia was checked by testing corneal reflexes and motor responses to tail pinch. Mean blood pressure and blood sample collection for gases

assessment were monitored through the catheterization of the femoral artery. Mice were artificially ventilated with a nitrogen/oxygen/CO₂ mixture through a tracheal intubation. Body temperature was maintained at 37 °C throughout the experiment. CBF was monitored with a laser-Doppler probe (AD Instruments, USA) placed stereotaxically on the thinned skull above the whisker barrel area of the somatosensory cortex. The flowmeter and blood pressure transducer were connected to a computerized data acquisition system (MacLab; Colorado Springs, CO). Analysis of CBF responses began 30 minutes after the end of the surgery to allow blood gases to stabilize. Animals with a mean arterial blood pressure under 60 mmHg and/or blood gases outside normal range (pH: 7.35-7.40; pCO₂: 33-45; pO₂: 120-140) were eliminated from the study. CBF responses to neuronal activity were evaluated by whiskers stimulations. Three whiskers stimulations sessions of one minute at 6 Hz were done on the contralateral side to CBF measurement. Three minutes resting periods were left between each stimulation. CBF values were acquired with the LabChart6 Pro software (v6.1.3, AD Instruments, USA). The percentage increase in CBF represents the peak CBF response relative to the resting CBF peak values during the 20 seconds before stimulations.

Superoxide anion production

Superoxide anion production was assessed by hydroethidine microfluorography as previously described⁴¹. Hydroethidine (dihydroethidium) is cell permeable and is oxidized to become the fluorophore ethidium bromide that intercalates in double-stranded DNA⁴². Mice were anesthetized with sodium pentobarbital (100 mg/kg body weight, CDMV) and perfused transcardially with PBS, pH 7.4. Brains were carefully isolated, frozen on dry ice and stored at -80°C until further analysis. Frozen brains were cut with a cryostat (20µm) and brain sections

were mounted on slides and stored at -20°C overnight. The slides were air dried at room temperature for 15 min followed by 15 min on a slide warmer set at 45°C. The slides were then immersed in a dihydroethidium (DHE) solution (2µM, MilliporeSigma) dissolved in PBS at 37°C for 2 min. The slides were rinsed in PBS for 5 min and dried on a slide warmer for 20 minutes before they were coverslipped with Fluoromount-G mounting medium (Southern Biotech, USA). Images were acquired using an epifluorescence microscope Leica DM2000 with the same acquisition parameters for all groups. Analysis of relative fluorescence intensity was done using the Image J software (version 1.53; National Institutes of Health). DHE results are expressed relative to the control group.

Statistical analysis

Data analysis was performed with GraphPad Prism software (version 7.0, La Jolla, USA) and results are presented as mean $\pm$ SEM. CBF responses to whiskers stimulations, resting CBF, superoxide anion production and systolic blood pressure analysis were evaluated with an ANOVA for factorial design with repeated measures followed by a Bonferroni post-test for multiple group comparisons. The dose-response whiskers stimulations curve, resting CBF and mean arterial pressure from supplemental Figure 1 were analysed using a one-way-ANOVA followed by Dunnet's post-test. Significance was set at $p < 0.05$. Sample size per group is presented in the results section as well as in the figure legends.

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Figures and Figure legends :

Figure 1: IL-17A neutralization prevents neurovascular coupling impairment induced by Ang II. Cerebral blood flow (CBF) in response to whiskers stimulations measured *in vivo* by laser Doppler flowmetry in C57BL/6 male mice treated with an anti-IL-17A neutralizing antibody (IL-17A Ab) or control mouse immunoglobulin G (IgG) antibody (0.5 $\mu\text{g}/\mu\text{L}$ per mouse every 4 days, i.p.). This administration started at day 0 of Ang II (600 ng/kg/min, 14 days) infusion through an osmotic minipump or sham surgery. The graphs depict (a) CBF as percentage changes with respect to the initial CBF value, (b) resting CBF value and (c, d) represent 1 second average curves of the evoked CBF, expressed in arbitrary unit. SEM is represented by the lighter tone shade surrounding each curve. Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test. ** $p < 0.01$, $n = 10-12$ per group.

Figure 2: IL-17A receptor inhibition prevents Ang II-induced neurovascular coupling

alteration. Cerebral blood flow (CBF) in response to whiskers stimulations measured *in vivo* by laser Doppler flowmetry in C57BL/6 male mice treated with an IL-17A receptor antagonist (IL-17RA mAB) or control mouse immunoglobulin G (IgG) antibody (0.5 $\mu\text{g}/\mu\text{L}$ per mouse every 4 days, i.p.). This administration started at day 0 of Ang II (600 ng/kg/min, 14 days) infusion through an osmotic minipump or sham surgery. The graphs depict (a) CBF as percentage changes with respect to the initial CBF value, (b) resting CBF value and (c, d) represent 1 second average curves of the evoked CBF, expressed in arbitrary unit. SEM is represented by the lighter tone shade surrounding each curve. Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test. ** $p < 0.01$, $n = 7-10$ per group.

Figure 3: IL-17A neutralization prevents Ang II-induced oxidative stress production.

Superoxide anion production quantified using dihydroethidium fluoromicrography (DHE) in C57BL/6 male mice treated with an anti-IL-17 neutralizing antibody (IL-17A Ab) or control mouse immunoglobulin G (IgG) antibody (0.5 $\mu\text{g}/\mu\text{L}$ per mouse every 4 days, i.p.). This administration started at day 0 of Ang II (600 ng/kg/min, 14 days) infusion through an osmotic minipump or sham surgery. The graphs depict the ratio of fluorescence compared to control mice in the (a) somatosensory cortex and different regions of the hippocampus: (b) lacunosum moleculare (LMol), (c) dentate gyrus (DG), (d) cornu ammonis 1 (CA1) and (e) cornu ammonis 3 (CA3). (f) Representative micrographs of DHE staining in CA1 of the hippocampus (50 μm scale). Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$; $n = 7-10$ per group.

Figure 4: IL-17A receptor inhibition prevents oxidative stress production induced by Ang

II. Superoxide anion production quantified using dihydroethidium (DHE) fluoromicrography (DHE) in C57BL/6 male mice treated with an IL-17A receptor antagonist (IL-17RA mAB) or control mouse immunoglobulin G (IgG) antibody (0.5 μ g/ μ L per mouse every 4 days, i.p.). This administration started at day 0 of Ang II (600 ng/kg/min, 14 days) infusion through an osmotic minipump or sham surgery. The graphs depict the ratio of fluorescence compared to control mice in the (a) somatosensory cortex and different regions of the hippocampus: (b) lacunosum moleculare (LMol), (c) dentate gyrus (DG), (d) cornu ammonis 1 (CA1) and (e) cornu ammonis 3 (CA3). (f) Representative micrographs of DHE staining in CA1 of the hippocampus (50 μ m scale). Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$; $n = 7-10$ per group.

Figure 5: Tempol treatment prevents the neurovascular coupling alteration induced by IL-17A. Cerebral blood flow (CBF) in response to whiskers stimulations measured *in vivo* by laser Doppler flowmetry in C57BL/6 male mice receiving IL-17A recombinant (Rb) through an osmotic minipump (50 pg/kg/h, 7 days) and treated with or without Tempol (1mM changed every 2 days) administered in the drinking water. The graphs depict (a) CBF as percentage changes with respect to the initial CBF value, (b) resting CBF value and (c, d) represent 1 second average curves of the evoked CBF, expressed in arbitrary unit. SEM is represented by the lighter tone shade surrounding each curve. Data were analysed using ANOVA for factorial design with repeated measures followed by a Bonferroni post-test. * $p < 0.05$, ** $p < 0.01$ $n = 7-8$ per group.

Figure 6: NOX-2 depletion prevents IL-17A-induced neurovascular coupling impairment.

Cerebral blood flow (CBF) in response to whiskers stimulations measured *in vivo* by laser Doppler flowmetry in NOX-2^{-/-} or WT C57BL/6 male mice receiving IL-17A recombinant (Rb) through an osmotic minipump (50 pg/kg/h, 7 days). The graphs depict (a) CBF as percentage changes with respect to the initial CBF value, (b) resting CBF value and (c, d) represent 1 second average curves of the evoked CBF, expressed in arbitrary unit. SEM is represented by the lighter tone shade surrounding each curve. Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test. * $p < 0.05$, $n = 7-9$ per group.

Figure 7: Tempol treatment prevents IL-17A-induced oxidative stress production.

Superoxide anion production quantified using dihydroethidium (DHE) fluoromicrography (DHE) in C57BL/6 mice receiving IL-17A Rb through an osmotic minipump (50 pg/kg/h, 7 days) and treated with or without Tempol (1mM changed every 2 days) administered in the drinking water. The graphs depict the ratio of fluorescence compared to control mice in the (a) somatosensory cortex and different regions of the hippocampus: (b) lacunosum moleculare (LMol), (c) dentate gyrus (DG), (d) cornu ammonis 1 (CA1) and (e) cornu ammonis 3 (CA3). (f) Representative micrographs of DHE staining in CA1 of the hippocampus (50 μ m scale). Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$; $n = 6-7$ per group.

Figure 8: NOX-2 depletion prevents oxidative stress production induced by IL-17A.

Superoxide anion production quantified using dihydroethidium (DHE) fluoromicrography (DHE) in NOX-2^{-/-} or WT C57BL/6 male mice receiving IL-17A Rb through an osmotic minipump (50 pg/kg/h, 7 days). The graphs depict the ratio of fluorescence compared to control in the (a) somatosensory cortex and different regions of the hippocampus: (b) lacunosum moleculare (LMol), (c) dentate gyrus (DG), (d) cornu ammonis 1 (CA1) and (e) cornu ammonis 3 (CA3). (f) Representative micrographs of DHE staining in CA1 of the hippocampus (50 μ m scale). Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$; $n = 8-10$ per group.

Supplemental Figure 1 : Effect of chronic IL-17A administration on neurovascular coupling. CBF responses to whiskers stimulations measured *in vivo* by laser Doppler flowmetry in C57BL/6 male mice receiving 12.5 to 100 pg/kg/h of IL-17A Rb through an osmotic minipump (7 days) or sham surgery. The graphs depict (a) CBF as percentage changes with respect to the initial CBF value, (b) the resting CBF value, or (c) the mean arterial pressure monitored simultaneously with CBF. Data were analysed using one-way-ANOVA followed by Dunnet's post-test. ** $p < 0.01$, *** $p < 0.001$ $n = 10-12$ per group.

Supplemental Figure 2: IL-17A neutralization partially prevents the increased systolic blood pressure induced by Ang II. Systolic blood pressure assessed at (a) day 0, (b) day 7 and (c) day 14 by tail-cuff plethysmography in C57BL/6 male mice treated with an anti-IL-17A neutralizing antibody (IL-17A Ab) or control mouse immunoglobulin G (IgG) antibody (0.5 $\mu\text{g}/\mu\text{L}$ per mouse every 4 days, i.p.). This administration started at day 0 of Ang II (600 ng/kg/min, 14 days) infusion through an osmotic minipump or sham surgery. Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test., *** $p < 0.001$ and **** $p < 0.0001$ $n = 11-18$ per group.

Supplemental Figure 3: IL-17A receptor inhibition partially prevents increased systolic

blood pressure induced by Ang II. Systolic blood pressure assessed at (a) day 0, (b) day 7 and

(c) day 14 by tail-cuff plethysmography in C57BL/6 male receiving an IL-17A receptor

antagonist (IL-17RA mAB) or control mouse immunoglobulin G (IgG) antibody (0.5 $\mu\text{g}/\mu\text{L}$ per

mouse every 4 days, i.p.). This administration started at day 0 of Ang II (600 ng/kg/min, 14 days)

infusion through an osmotic minipump or sham surgery. Data were analysed using ANOVA for

factorial design followed by a Bonferroni post-test. ** $p < 0.01$ and **** $p < 0.0001$ $n = 9-13$ per

group.

Supplemental Figure 4: Effect of NOX-2 depletion on systolic blood pressure increase induced by chronic IL-17A Rb administration. Systolic blood pressure assessed at (a) day 0 and (b) day 7 by tail-cuff plethysmography in NOX-2^{-/-} or WT C57BL/6 male mice receiving IL-17A Rb through an osmotic minipump (50 pg/kg/h, 7 days). Data were analysed using ANOVA for factorial design followed by a Bonferroni post-test. *** $p < 0.001$, **** $p < 0.0001$ n=8-10 per group.