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Sensibilité à la douleur, fonction olfactive et plasticité cérébrale chez un modèle murin de cécité congénitale

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Résumé

La cécité précoce induit des changements comportementaux souvent accompagnés par des changements fonctionnels et neuroanatomiques au niveau du cerveau. Alors que les modifications dans les modalités tactiles et auditives ont été largement étudiées, les changements touchant l'olfaction et la douleur chez les aveugles sont restés moins explorés. Chez l'humain aveugle précoce, certaines études ont rapporté une amélioration de la fonction olfactive alors que d'autres n'ont pas réussi à démontrer de tels effets. Chez l'humain, des études récentes ont mis en évidence une hypersensibilité à la douleur aiguë chez les aveugles précoces. Cependant, les mécanismes sous-jacents sont restés inconnus. Afin d'étudier les changements olfactifs et nociceptifs induits par la cécité précoce ainsi que la plasticité fonctionnelle et neuroanatomique qui les accompagne, nous avons développé un modèle de souris de cécité précoce appelé ZRDBA. Dans cette souche, en croisant un parent homozygote pour le gène Rax/Rx (gène responsable de l'anophtalmie) avec un parent hétérozygote, dans une même portée la moitié des souris naissent anophtalmes alors que l'autre moitié a une vue normale. Cette souche nous permet d'examiner les modifications comportementales et cérébrales induites par la cécité chez deux groupes de souris ayant la même base génétique.

Le premier objectif de cette thèse était d'évaluer les changements comportementaux olfactifs induits par la cécité chez les souris ZRDBA et d'examiner si ces changements sont accompagnés de plasticité anatomique dans les régions cérébrales impliquées dans le traitement olfactif. Trois tests comportementaux ont été menés : le test de recherche de nourriture, le test du seuil olfactif et le test de performance olfactive. Les résultats ont révélé des meilleures performances olfactives chez les aveugles dans le test de recherche de nourriture ainsi que dans le test de performance olfactive, mais pas dans le test du seuil olfactif. Ces résultats indiquent une amélioration de la discrimination et identification des odeurs chez les souris aveugles. La plasticité cérébrale dans les structures olfactives a été examinée par des analyses histologiques et analyses IRM. Les résultats des mesures histologiques ont révélé une augmentation du volume des bulbes olfactifs, premier relais de traitement des informations olfactives, chez les souris aveugles. Les analyses IRM ont révélé une augmentation du volume dans les couches granulaires et glomérulaires des bulbes olfactifs ainsi que dans d'autres régions impliquées dans

le traitement olfactif, notamment, le cortex orbitofrontal et le cortex piriforme. Ces résultats suggèrent que l'amélioration de la fonction olfactive chez les souris aveugles peut être expliquée par la plasticité anatomique mise en évidence dans les structures olfactives.

Le deuxiéme objectif de cette thèse était d'évaluer la sensibilité à la douleur chez les souris aveugles ZRDBA. Quatre tests nociceptifs ont été réalisés : le test de formaline (sensibilité chimique), le test de Von Frey (sensibilité mécanique), le test d'acétone (sensibilité au froid) et le test de tail-flick dans l'eau (sensibilité au chaud). Les souris aveugles, lorsque comparées à leurs congénères voyantes, ont montré une hypersensibilité à la douleur dans tous les tests. Afin d'examiner les mécanismes sous-jacents de cette hypersensibilité, nous avons investigué par le biais d'analyses immunohistologiques la plasticité fonctionnelle et anatomique dans l'amygdale, structure clé pour la modulation et traitement de la douleur. Les résultats ont montré une augmentation de l'activité c-fos induite par l'injection de la formaline dans le noyau central de l'amygdale et dans toute l'amygdale chez les souris aveugles. Les analyses histologiques ont également montré une augmentation du volume de l'amygdale chez les souris aveugles. Ces résultats suggèrent la contribution de l'amygdale dans l'hypersensibilité à la douleur mise en évidence chez les souris aveugles.

Finalement, dans la troisième partie de cette thèse, nous avons voulu investiguer l'impact de la cécité sur la plasticité dans l'ensemble du cerveau à l'aide d'analyses IRM et d'analyses histologiques. Les résultats de cette étude ont révélé une atrophie de la plupart des structures visuelles, notamment, le corps géniculé latéral, le cortex visuel primaire, le cortex visuel secondaire ainsi que les collicules supérieurs. En outre, les analyses histologiques ont révélé une atrophie de la couche IV dans le cortex visuel primaire et dans le cortex visuel secondaire ainsi qu'une atrophie des couches visuelles superficielles des collicules supérieurs chez les souris aveugles expliquant la réduction du volume observée dans ces régions. Dans les autres structures non visuelles, les analyses ont révélé une augmentation du volume dans l'amygdale, impliquée dans la douleur ainsi que dans plusieurs régions olfactives comme les bulbes olfactifs, le cortex piriforme et le cortex orbitofronal chez les souris aveugles. Ces résultats permettent de faire le parallèle avec les études réalisées chez l'humain et ouvrent la porte pour plus d'investigations des mécanismes sous-jacents de la plasticité cérébrale observée chez les aveugles.

Mots-clés : cécité, plasticité, olfaction, bulbes olfactifs, douleur, amygdale, ZRDBA.

Abstract

Early blindness induces behavioral changes often accompanied by functional and neuroanatomical changes in the brain. While changes in tactile and hearing modalities have been largely investigated, changes in olfaction and pain in the blind remained less explored. Some studies reported an improvement in olfactory function in early blind humans while others failed to demonstrate such effects. In addition, recent studies evidenced hypersensitivity to acute pain in early blind humans. However, the underlying mechanisms remained unknown. In order to study changes induced by early blindness in olfactory and nociceptive functions and their underlying functional and neuroanatomical plasticity, we developed a mouse model of early blindness called ZRDBA. In the unique ZRDBA strain, half of the mice homozygous for the Rax / Rx gene (gene responsible for anophthalmia) are born anophthalmic while the other half heterozygous are born sighted. ZRDBA mouse strain allows investigation of the behavioral and cerebral impacts of early blindness without worrying about strain differences.

The first aim of this thesis was to assess olfactory behavioral changes induced by blindness in ZRDBA mice and examine whether these changes are accompanied by anatomical plasticity in brain regions involved in olfactory processing. Three behavioral tests were conducted: the buried food test, the odor detection threshold test (sensitivity measure) and the olfactory performance test (three-odor discrimination measure). The results revealed better olfactory performance of blind mice in the buried food test as well as in the olfactory performance test but not in the olfactory threshold test. These results indicate an improvement in olfactory discrimination and identification in blind mice. Brain plasticity in olfactory structures was examined by histological and MRI analyses. The results of the histological measurements revealed a larger volume of the olfactory bulbs, the first site for processing olfactory information, in blind mice. MRI analysis revealed a larger volume in the granular and glomerular layers of the olfactory bulbs as well as in other regions involved in olfactory processing, namely, the orbitofrontal cortex and the piriform cortex. These results suggest that plasticity in the olfactory structures may underlie the improved olfactory function in blind mice.

The second aim of this thesis was to assess pain sensitivity in the blind ZRDBA mice. Four nociceptive tests were carried out: the formalin test (chemical sensitivity), the Von Frey

test (mechanical sensitivity), the acetone test (cold sensitivity) and the water tail-flick test (hot pain sensitivity). Blind mice showed hypersensitivity to pain in all tests. In order to examine the underlying mechanisms of this pain hypersensitivity, we investigated the functional and anatomical plasticity in the amygdala, a key structure for the modulation and treatment of pain using immunohistological analyses. The results revealed an increase of c-Fos activity induced by the injection of formalin in the central nucleus of the amygdala as well as the whole amygdaloid complex in blind mice. Histological measurement also revealed a larger volume of the amygdala in blind mice. These results suggest the contribution of the amygdala in pain hypersensitivity evidenced in blind mice.

Finally, in the third part of this thesis, we wanted to investigate the impact of blindness on anatomical plasticity in the whole brain using MRI and histological analyses. The results of this study revealed an atrophy of several visual structures, in particular, the lateral geniculate nucleus, the primary visual cortex, the secondary visual cortex as well as the superior colliculi. Moreover, histological analyses revealed an atrophy of layer IV of the primary visual cortex and the secondary visual cortex as well as atrophy of the superficial visual layers of the superior colliculus in blind mice which explains the volumetric reduction observed in these regions. In the non-visual structures, analyses revealed a larger volume in the amygdala, as well as in several olfactory structures such as the olfactory bulbs, the piriform cortex and the orbitofronal cortex in blind mice. These results contribute to the understanding of the impacts of early blindness on brain plasticity.

Keywords : Blindness, plasticity, olfaction, olfactory bulbs, pain, amygdala, ZRDBA.

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2MB 2-méthylbutyricacid

BLA Noyau basolatéral de l'amygdale

BLV Noyau basolatéral ventral de l'amygdale

CeA Noyau central de l'amygdale

CeL Noyau latéral de l'amygdale

CeLc Noyau capsulaire de l'amygdale

CeM Noyau médial de l'amygdale

DLG Noyau géniculé dorsolatéral

ERK Extracellular signal-regulated kinase

IASP International association for the study of pain

IRM Imagerie par résonance magnétique

IRMf Imagerie par résonance magnétique fonctionnelle

MeA Noyau médial de l'amygdale

NS Neurones spécifiques de la nociception

PAG Substance grise périaqueducale

PB Noyau parabrachial

TEP Tomographie à émission de positron

TMS Stimulation magnétique transcranienne

UPSIT Test de l'odorat de l'université de Pennsylvanie

V1 Cortex visuel primaire

V2 Cortex visuel secondaire

WDR Neurones à large gamme dynamique

Liste des abréviations

fig Figure

g Gramme

mm Millimètre

µL Microlitre

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Avant-propos

Les travaux de mon doctorat visaient à étudier les changements comportementaux et la plasticité cérébrale conséquents de la cécité précoce. Pour ce faire, nous avons développé un modèle de souris de cécité congénitale que nous avons nommé ZRDBA. Nous nous sommes focalisés sur les modifications comportementales de la fonction olfactive et de la perception de la douleur chez ce modèle de souris étant donné que ces deux modalités sont les moins investiguées chez les aveugles précoces. Nous avons également examiné, par le biais d'analyses d'imagerie par résonance magnétique et d'analyses histologiques, la plasticité cérébrale accompagnant les changements comportementaux observés. Les résultats de ces études ont mené à l'élaboration de trois articles scientifiques représentant trois chapitres distincts dans le présent ouvrage.

Au cours de mon doctorat, j'ai également été intégrée dans un projet visant à étudier plasticité cérébrale accompagnant l'hypersensibilité à la douleur chez un modèle de rat de douleur chronique inflammatoire du dos. Dans cette étude des analyses electrophysiologiques et histologiques ont été employées. L'article décrivant les résultats de cette étude est retrouvé en annexe étant donné qu'il s'intégrait difficilement au thème principal du présent ouvrage.

"Now there was among the Thebans a soothsayer, Tiresias, son of Everes and a nymph Chariclo, of the family of Udaeus, the Spartan, and he had lost the sight of his eyes ... Pherecydes says that he was blinded by Athena; for Chariclo was dear to Athena... and Tiresias saw the goddess stark naked, and she covered his eyes with her hands, and so rendered him sightless. And when Chariclo asked her to restore his sight, she could not do so, but by cleansing his ears she caused him to understand every note of birds; and she gave him a staff of cornel-wood, wherewith he walked like those who see." (Apollodorus, C2nd A.D).

Chapitre I Introduction et contexte général

I.1 Préambule

Le cerveau se forme et se construit selon une base génétique préexistante et il est par la suite, modelé selon les expériences vécues et les interactions avec l'environnement. Il a une capacité impressionnante de changer et de s'adapter. Dans les cas de perte sensorielle comme l'anosmie, la surdité ou la cécité, le cerveau s'adapte et plusieurs modifications ont lieu. Ces modifications cérébrales sont souvent accompagnées de changements comportementaux. Les conséquences de telles pertes sensorielles complètes ont intéressé les chercheurs et les philosophes depuis des siècles. Aristote a discuté dans son traité *De Sensu et Sensibilibus* le rôle de la vision et de l'audition dans l'apprentissage et comment leur absence pouvait affecter la capacité d'acquisition des connaissances chez les personnes aveugles et sourdes (Beare, 1931; Bock and Fine, 2014; Kupers and Ptito, 2014). Le philosophe français Denis Diderot (1749) a discuté dans son ouvrage *Lettre sur les aveugles à l'usage de ceux qui voient* les capacités sensorielles exceptionnelles que développent les aveugles afin de compenser la perte de vision. William James (1890) a émis l'hypothèse que les aveugles développent un sixième sens, une forme de perception tridimensionnelle caractérisée par la détection de changement de la pression dans l'air, qui leur permet d'éviter les obstacles.

Ces hypothèses basées sur des observations datant de plusieurs siècles ont été investiguées par la science moderne. Plusieurs chercheurs ont étudié les performances des aveugles dans les modalités sensorielles non visuelles. Si certaines études n'ont pas réussi à y trouver des différences fonctionnelles entre aveugles et voyants (Heller, 1989; Kolarik, et al., 2014; Sorokowska, et al., 2018; Sorokowska, et al., 2019), d'autres ont pu confirmer que les aveugles de naissance ou les aveugles ayant perdu la vue à un âge précoce ont des meilleures performances dans les tâches non visuelles. Ces études ont montré que, comparés aux voyants, les aveugles sont meilleurs en navigation spatiale (Fortin, et al., 2008), ont une meilleure acuité tactile (Goldreich and Kanics, 2003; Wong, et al., 2011) ainsi que des meilleures performances auditives (Wan, et al., 2010; King, 2015). Une équipe canadienne a également investigué l'impact de la cécité sur le système nociceptif. Dans leur étude, ils ont montré que les aveugles

congénitaux ont une sensibilité à la douleur supérieure aux personnes voyantes (Slimani, et al., 2013). En ce qui concerne l'olfaction, les résultats des quelques études réalisées n'étaient pas toujours concluants et s'avéraient non congruents. Certaines études indiquent que les aveugles ont des meilleures performances olfactives (Cuevas, et al., 2009; Rombaux, et al., 2010; Comoglu, et al., 2015; Gagnon, et al., 2015a; Iversen, et al., 2015; Araneda, et al., 2016); tandis que d'autres n'ont pas réussi pas à démontrer cet effet (Schwenn, et al., 2002; Beaulieu-Lefebvre, et al., 2011). En outre, une méta-analyse récente par Sorokowska et al. (2018) a montré qu'il y avait un biais dans la plupart des études qui ont montré la supériorité des aveugles dans les tâches olfactives. D'où l'importance de développer des modèles animaux pour examiner la perception olfactive avec des tests plus ciblés et contrôlés.

Les adaptations comportementales sont accompagnées de changements structurels et fonctionnels dans le cerveau. Cette plasticité mise en évidence par des techniques d'imagerie dépasse les structures visuelles et touche plusieurs structures cérébrales (Kupers and Ptito, 2014). Ces études ont montré des changements dans les connexions cortico-corticales et sous-corticales ainsi que des modifications de volume et d'épaisseur des structures cérébrales visuelles et non visuelles (Bock and Fine, 2014; Kupers and Ptito, 2014). Cependant, les résultats des études de plasticité ne sont pas toujours concourants et les mécanismes sous-jacents aux modifications des modalités sensorielles chez les aveugles ne sont pas connus. D'où l'importance du développement de modèles animaux valides qui permettent l'investigation approfondie de ces aspects.

Dans cette thèse, nous avons utilisé un modèle de souris anophtalme, récemment développé dans notre laboratoire. Dans cette nouvelle souche appelée ZRDBA la moitié des souris naît aveugle et l'autre moitié, voyante, permettant ainsi d'évaluer les impacts directs de la cécité en comparant deux groupes de souris de la même souche avec les mêmes caractéristiques génétiques.

Le premier objectif de cette thèse était d'examiner les performances olfactives chez le modèle de souris aveugle congénital par différents tests comportementaux et d'investiguer par des techniques d'imagerie et d'histologie, si les changements comportementaux olfactifs sont accompagnés d'une plasticité dans les structures cérébrales olfactives afin de déterminer les mécanismes sous-jacents probables. Le deuxième objectif était d'évaluer la sensibilité à la

douleur et d'investiguer les mécanismes sous-jacents à l'hypersensibilité aux stimuli nociceptifs chez les souris aveugles en examinant les changements structurels et fonctionnels dans l'amygdale, région impliquée dans le traitement et la modulation de la douleur. Enfin, nous avons examiné les impacts de la cécité précoce sur la plasticité des différentes structures visuelles et non visuelles chez le modèle de souris aveugle par des techniques d'imagerie structurelle.

I.2 Impacts de la cécité

En premier lieu, les impacts comportementaux de la cécité seront abordés, nous nous pencherons par la suite, sur la plasticité cérébrale qui accompagne ces adaptations comportementales.

I.2.1 Période critique de développement : Aveugles précoces versus aveugles tardifs

Avant de discuter les impacts comportementaux, structurels et fonctionnels de la cécité, il est important de faire la différence entre les aveugles précoces et les aveugles tardifs.

Cécité précoce

La cécité est dite précoce si elle survient avant la fin de la période critique de plasticité. La période critique est la période durant laquelle le système est capable de s'adapter à des stimulations ou des changements dans l'environnement. Elle est différente d'un système à l'autre et même à l'intérieur d'un même système, les périodes critiques sont différentes selon les niveaux – cortical, sous-cortical – et la fonction. Elle varie également selon l'espèce. Les individus aveugles congénitaux et aveugles précoces sont souvent groupés dans les études dans un même groupe d'aveugles précoces.

Aveugles congénitaux: fait référence aux individus nés aveugles et qui n'ont jamais été exposés à des stimuli visuels (Voss, 2013).

Aveugles précoces : fait référence aux cas de cécité qui surviennent dans les premières 2 à 5 années chez l'humain (Chebat, et al., 2007; Voss, 2013; Anurova, et al., 2015; Maller, et al., 2016) et premier jour chez la souris (Chabot, et al., 2007).

Cécité tardive

La cécité est dite tardive si elle survient après la fin de la période critique de plasticité.

Aveugles tardifs: Fait référence aux cas de cécité survenant entre les premières années de la vie (en général après l'âge de 7 ans) jusqu'à l'âge adulte (Voss, 2013).

Il y a une grande variabilité dans les études chez les humains dans la définition de l'âge précoce et tardif de la cécité. Certaines études incluent des sujets ayant perdu la vue jusqu'à l'âge de 14 ans dans le groupe aveugles précoces (Voss, 2013). Ce manque de consistance dans la définition des groupes d'aveugles présente un biais important dans l'échantillonnage et contribue à la grande variabilité des résultats entre les différentes études (Voss, 2013). Par exemple, inclure des aveugles tardifs dans des groupes d'aveugles précoces pose un biais important dans les études où on essaie d'associer l'âge de la cécité à des changements comportementaux ou neuroanatomiques (Voss, 2013). Ceci peut également induire un chevauchement entre les groupes expérimentaux dans les différentes études, où un individu donné peut être considéré comme aveugle précoce dans une étude alors qu'il est considéré comme aveugle tardif dans une autre (Voss, 2013).

Dans les parties qui suivent, les impacts comportementaux et cérébraux de la cécité précoce seront discutés.

I.2.2 Impacts comportementaux de la cécité

Diderot (1749) a mentionné dans son ouvrage *Lettre sur les aveugles* le cas du célèbre mathématicien aveugle Saunderson qui pouvait reconnaître les fausses pièces d'argent par le toucher. Diderot a ensuite suggéré que les personnes aveugles de naissance peuvent développer des capacités exceptionnelles afin de compenser la perte de vision. Mythe ou réalité?

Pour répondre à cette question, plusieurs chercheurs ont investigué les changements comportementaux concernant les traitements tactile, auditif, olfactif et nociceptif suite à la privation visuelle.

I.2.2.1 Traitement tactile

Parmi les premières études à évaluer les performances tactiles chez les aveugles précoces, l'étude de Craig (1988). Ce dernier, a observé une meilleure performance dans la reconnaissance des lettres par les doigts chez les aveugles. Une étude subséquente menée par Van Boven et collaborateurs (2000) a montré que les aveugles précoces avaient une meilleure discrimination de l'orientation des lettres comparés aux personnes voyantes. D'autres études ont suivi et ont montré également une meilleure acuité tactile chez les aveugles précoces lecteurs de Braille comparés aux voyants contrôles (Goldreich and Kanics, 2003; Alary, et al., 2008; Legge, et al., 2008). Dans certaines de ces études, il n'y avait pas de corrélation entre l'âge du début la cécité, la vitesse de lecture Braille et la performance tactile (Goldreich and Kanics, 2003; Legge, et al., 2008) supportant l'hypothèse que cette acuité améliorée est indépendante de l'expérience tactile et est directement reliée à la perte de vision (Goldreich and Kanics, 2003). Par ailleurs, dans certaines études, on n'a pas réussi à observer de différences significatives dans le seuil, sensibilité tactile, ou détection des vibrations entre les aveugles et les voyants en rapportant que l'avantage des aveugles disparait quand les voyants reçoivent un entrainement additionnel (Heller, 1989; Grant, et al., 2000). Wong et al., (2011) ont testé si les meilleures performances tactiles chez les aveugles seraient la conséquence directe de la perte de vision ou si elles seraient plutôt associées à l'expérience tactile. Dans cette étude, les auteurs ont testé la discrimination tactile de l'orientation d'un réseau sur l'index, le majeur et l'annulaire de la main dominante et non dominante ainsi que les lèvres sur un large groupe d'aveugles avec différents degrés d'expérience de lecture en Braille ainsi que sur des voyants. Les résultats ont montré que les participants aveugles surpassaient les voyants au niveau des doigts, mais pas des lèvres. En outre, les aveugles ayant le plus d'expérience de lecture Braille avaient une meilleure performance avec leurs doigts utilisés pour la lecture Braille qu'avec les autres doigts. De plus, cette acuité tactile améliorée corrélait avec le degré d'expérience de lecture Braille (Wong, et al., 2011). Une autre étude réalisée par Alary et al. (2009) a montré que les aveugles lecteurs de Braille avaient une meilleure performance comparés aux voyants dans les tâches de discrimination de textures semblables aux textures des lettres Braille mais pas dans les tâches de discrimination des vibrations. Ces résultats supportent l'hypothèse que l'amélioration des

performances tactiles observées chez les aveugles est plutôt le résultat d'un entrainement et expérience tactiles accrus (Alary, et al., 2009; Wong, et al., 2011).

Des expériences ciblées et plus élaborées pourraient mieux expliquer l'interaction entre la privation visuelle et l'entrainement pour l'amélioration des performances tactiles. Cela pourrait par exemple être directement testé en comparant des sujets voyants qui sont intensivement entraînés et avec les yeux bandés pendant des périodes prolongées avec des individus qui sont soit uniquement entraînés soit uniquement les yeux bandés. De telles expériences aideraient à déterminer si la privation sensorielle augmente effectivement la performance ou ne fait qu'accélérer les effets de l'entraînement (Voss, et al., 2014).

I.2.2.2 Traitement auditif

Plusieurs études ont examiné le traitement auditif chez les aveugles. Les études réalisées n'ont pas réussi à montrer une différence dans le seuil auditif entre les aveugles et les voyants (Collignon, et al., 2006; Collignon and De Volder, 2009; Nilsson and Schenkman, 2016). D'autres études ont montré que les aveugles précoces ont une meilleure discrimination du ton vocal et musical comparés aux voyants (Gougoux, et al., 2004; Hamilton, et al., 2004; Wan, et al., 2010). Dans l'étude réalisée par Hamilton et al. (2004), les aveugles précoces étaient capables d'identifier des tonalités musicales très spécifiques, que seuls des musiciens expérimentés pouvaient identifier. Wan et al. (2010) ont comparé la discrimination et la catégorisation des tonalités musicales chez les aveugles congénitaux, les aveugles tardifs et un groupe de voyants contrôles. Les résultats indiquent que les aveugles congénitaux ont une meilleure performance que les aveugles tardifs et les voyants dans la discrimination et la catégorisation de tonalité (Wan, et al., 2010). Les aveugles précoces montrent également, une meilleure capacité de localisation sonore. Ceci a été démontré chez les animaux aveugles (Rauschecker and Kniepert, 1994) ainsi que chez les humains aveugles (Lessard, et al., 1998; Roder, et al., 1999; Fieger, et al., 2006). Des études subséquentes d'imagerie ont permis de montrer les modifications cérébrales sous-jacentes de ces résultats comportementaux. Deux études utilisant la tomographie à émission de positron (TEP) (Gougoux, et al., 2005; Voss, et al., 2008) ont montré que pendant le test de localisation sonore, les aveugles précoces montrent une activation du cortex occipital témoignant d'une plasticité intermodale induite par la cécité.

Cette activation était absente chez les aveugles tardifs et les voyants. Ces données suggèrent que l'activation du cortex occipital est étroitement liée aux capacités auditives améliorées chez cette population. Il y a cependant quelques études qui ne montrent pas de différence de performance dans la localisation sonore entre aveugles et voyants. Par exemple, dans une étude réalisée par Zwiers et al. (2001), les auteurs ont testé la localisation sonore chez les aveugles et les voyants avec une source de son frontale (variant en hauteur). Les résultats suggèrent l'absence de différences de performance entre les aveugles et les voyants. Les aveugles avaient même une performance inférieure aux voyants dans l'extraction des indices spectraux liés à l'altitude dans l'environnement acoustique (Zwiers, et al., 2001). Cependant, bien que les aveugles aient des performances inférieures aux voyants dans la localisation sonore en région frontale, ils peuvent être meilleurs dans la localisation en d'autres régions (latérale, postérieure..).

En conclusion, la plupart des études indiquent que les aveugles ont une meilleure identification et catégorisation auditives que les personnes voyantes. Comme pour la modalité tactile, des expériences ciblées aideraient à déterminer si ces effets sont le résultat direct de la privation visuelle ou si elles sont la conséquence d'une utilisation accrue de la modalité auditive.

I.2.2.3 Traitement olfactif

Seuil olfactif

Le seuil olfactif est défini comme la plus basse concentration à laquelle la présence d'un odorant est détectée de manière fiable (Stevens, 1960). Historiquement, le seuil olfactif a été la première fonction olfactive examinée chez les aveugles. Les premières études réalisées (Griesbach, 1899; Cherubino and Salis, 1957; Boccuzzi, 1962) n'ont pas rapporté de différences entre les aveugles et les voyants. Appuyant ces résultats, la plupart des études plus récentes qui ont également examiné le seuil olfactif chez les aveugles, ont montré des performances similaires entre les aveugles et les voyants (Smith, et al., 1993; Luers, et al., 2014; Sorokowska, 2016). Seulement trois études ont montré un seuil olfactif plus bas chez les aveugles (Cuevas, et al., 2010; Beaulieu-Lefebvre, et al., 2011; Comoglu, et al., 2015) et d'autres un seuil olfactif plus haut (Murphy and Cain, 1986). Globalement, les résultats de la plupart des études suggèrent l'absence de différence de seuil olfactif entre les aveugles et les voyants. Cependant, certaines des études citées plus haut ainsi que d'autres suggèrent des meilleures performances des

aveugles dans les tâches de discrimination et identification. La tâche du seuil olfactif est une tâche qui semble dépendre des mécanismes olfactifs périphériques et qui, contrairement aux tâches de discrimination et identification des odeurs, discutés dans la suite, ne nécessite pas un traitement cognitif complexe (Hedner, et al., 2010; Whitcroft, et al., 2017; Sorokowska, et al., 2018).

Discrimination et identification des odeurs

Le test de la discrimination olfactive consiste à présenter aux participants 2 ou 3 odeurs et leur demander si les odeurs sont différentes s'il s'agit de 2 odeurs ou à repérer l'odeur différente s'il s'agit de 3 odeurs. Comme pour le seuil olfactif, les résultats des études ne sont pas toujours concordants. Tandis que certaines études n'ont pas réussi à montrer des différences entre les aveugles et les voyants dans la discrimination des odeurs (Beaulieu-Lefebvre, et al., 2011; Manescu, et al., 2018), d'autres ont montré de meilleures performances chez les aveugles (Cuevas, et al., 2009; Cuevas, et al., 2010; Rombaux, et al., 2010; Comoglu, et al., 2015). Dans le test d'identification des odeurs, les participants doivent identifier l'odeur présentée soit à partir d'une liste de choix soit librement (sans choix préétablis). En ce qui concerne l'identification des odeurs, les aveugles sont meilleurs quand il s'agit de tâches plus compliquées comme l'identification libre des odeurs (Rosenbluth, et al., 2000; Wakefield, et al., 2004; Cuevas, et al., 2009; Cuevas, et al., 2010; Renier, et al., 2013; Gagnon, et al., 2015b), mais il n'y a pas de différence quand il s'agit d'identification avec des choix préétablis (Smith, et al., 1993; Rosenbluth, et al., 2000; Beaulieu-Lefebvre, et al., 2011). Une autre étude a montré que les aveugles sont meilleurs dans l'identification des odeurs à valence négative comme les odeurs de sueurs (Iversen, et al., 2015). Le nombre d'études réalisées n'est cependant pas suffisant pour tirer une conclusion claire sur la supériorité olfactive des aveugles. En outre, une méta-analyse récente a montré qu'il n'existe pas de différence de perception olfactive entre les aveugles et les voyants et qu'il existe un biais dans la plupart des études montrant des meilleures performances chez les aveugles (Sorokowska, et al., 2018). Les études incluses dans la méta-analyse ne sont pas nombreuses en plus d'être très hétérogènes avec des tailles de groupes variables, différents degrés de déficience visuelle, âge de cécité variable ainsi que d'importantes différences interindividuelles (Sorokowska, et al., 2018). L'effet de la cécité sur l'olfaction n'est

pas direct et peut être influencé par d'autres facteurs (environnement, activités quotidiennes...Etc.) à part la privation visuelle. Ceci souligne l'importance de tester ces aspects sur des modèles animaux de cécité précoce qui permettraient d'avoir un plus grand nombre d'échantillons ainsi que de contrôler les facteurs environnementaux afin d'avoir les mêmes conditions d'élevage pour les souris aveugles et voyantes. Une seule étude a été réalisée chez l'animal par Zhou et al., (2017) qui a montré qu'une privation visuelle postnatale chez la souris, née voyante, résulte en une amélioration de la discrimination olfactive. Dans la présente thèse, un modèle animal de cécité congénitale a été développé afin de comparer la fonction olfactive entre des souris aveugles et des souris voyantes disposant des mêmes caractéristiques génétiques et élevées dans les mêmes conditions.

Plasticité liée aux performances olfactives chez les aveugles

Les résultats des études suggèrent qu'en général, les aveugles comptent plus sur l'olfaction comparés aux voyants pour l'identification et la discrimination des éléments dans leur environnement. Ces performances olfactives améliorées peuvent être expliquées par une plasticité neuroanatomique mise en évidence dans plusieurs études. Chez les aveugles congénitaux, une activation du cortex occipital a été mise en évidence pendant la réalisation de tâches olfactives (Kupers, et al., 2011). Renier et al. (2013) ont montré que l'activation du cortex occipital pendant les tâches olfactives est corrélée avec des performances olfactives supérieures chez les aveugles. En outre, Rombaux et al. ont rapporté que la performance olfactive améliorée chez les aveugles congénitaux est corrélée avec une augmentation de la taille des bulbes olfactifs (Rombaux, et al., 2010). Chez les souris, il a été montré que l'amélioration des performances olfactives suite à une courte privation visuelle postnatale est accompagnée d'une augmentation de l'activité fonctionnelle dans les bulbes olfactifs et le cortex piriforme (Zhou, et al., 2017). Les bulbes olfactifs sont en effet, le premier relais pour le traitement de l'information olfactive (Mori, et al., 1999). De plus, plusieurs études ont montré que les bulbes olfactifs sont très plastiques (Huart, et al., 2013) et que leur taille corrèle avec la performance olfactive (Buschhuter, et al., 2008; Seubert, et al., 2013; Mazal, et al., 2016). Le volume des bulbes olfactifs peut augmenter suite à l'entraînement (Huart, et al., 2013) et décroître dans le

cas de troubles olfactifs (Yousem, et al., 1999; Mueller, et al., 2005; Rombaux, et al., 2006a; Rombaux, et al., 2006b).

Afin d'élucider l'impact de la cecité sur la perception olfactive ainsi que sur la plasticité des structures impliquées dans la fonction olfactive, dans la première étude de cette thèse, nous avons investigué les performances olfactives chez les souris aveugles ZRDBA. Par la suite, nous avons examiné la plasticité structurelle dans les bulbes olfactifs accompagnant ces modifications comportementales olfactives.

Objectif 1: Investiguer les performances olfactives et la plasticité anatomique des bulbes olfactifs chez les souris aveugles.

Hypothèse 1: Les souris aveugles auraient des meilleures performances olfactives comparées aux souris voyantes. Cette amélioration comportementale serait accompagnée par une plasticité anatomique des bulbes olfactifs.

I.2.2.4 Traitement nociceptif

Avant de discuter les impacts de la cécité sur la perception de la douleur, il est important de définir la douleur, qui diffère dans sa nature et dans sa complexité des autres modalités sensorielles discutées plus haut.

La définition officielle de la douleur selon l'IASP (International Association for the Study of Pain): *La douleur est une expérience sensorielle et émotionnelle désagréable associée à une lésion tissulaire réelle ou potentielle, ou décrite en termes de telles lésions.*

La douleur est une expérience neuropsychologique complexe associant des composantes sensorielles et affectives (Melzack and Casey, 1968). Le traitement de la douleur implique donc différentes structures cérébrales. Par conséquent, les changements de perception de la douleur survenant suite à la perte visuelle diffèrent dans leur nature et leurs mécanismes sous-jacents des autres adaptations sensorielles (olfactives, auditives et tactiles).

La première étude qui a décrit l'effet de la privation visuelle sur la perception de douleur a été réalisée par Zubek et al. (1964) qui ont montré qu'une privation visuelle (yeux bandés) d'une durée de 7 jours chez des personnes voyantes résulte en une baisse du seuil de douleur. Cette augmentation de la sensibilité à la douleur a persisté plusieurs semaines après l'expérience (Zubek, et al., 1964). Dans des études plus récentes, le traitement nociceptif thermique chez les aveugles congénitaux a été évalué par l'équipe de Slimani et al. (2013; 2014; 2015). Leur premier test consistait à appliquer un stimulus thermique à l'aide d'un laser sur la main dominante du participant et évaluer le seuil de douleur pour les deux groupes testés, soit les aveugles congénitaux et les voyants (Slimani, et al., 2013). Les résultats ont montré que les aveugles congénitaux ont un seuil de douleur plus bas et ont évalué les stimuli thermiques comme étant plus douloureux comparés à leurs homologues voyants (Slimani, et al., 2013). Dans la 2ème étude réalisée par la même équipe, le même paradigme expérimental a été reproduit en introduisant un groupe d'aveugles tardifs. Les résultats ont montré que les aveugles tardifs, contrairement aux aveugles congénitaux, ne montrent pas de signes d'hypersensibilité à la douleur et ont des seuils et scores de douleur comparables aux voyants (Slimani H, 2014). Toutefois, les mécanismes sous-jacents de cette hypersensibilité à la douleur restent inconnus. Cette derniére pourrait être induite par une plasticité dans les structures cérébrales impliquées dans le traitement de la douleur.

Dans la suite, une description des différentes afférences nociceptives ainsi que le rôle de l'amygdale, structure clé dans le traitement et la modulation de la douleur, seront discutées.

I.2.2.5 Afférences nociceptives

Les fibres afférentes sensorielles sont classées selon leurs structures, diamètres et vélocités en trois groupes majeurs : groupe A, B et C. Les fibres du groupe A sont myélinisées et sont subdivisées en quatre sous-groupes (Guyton and Hall, 1986) :

Aα : regroupe les fibres musculaire Ia et Ib et conduisent les informations proprioceptives.

Aβ : regroupe les mécanorécepteurs à bas seuil ainsi que les fibres afférentes des récepteurs de l'étirement. En circonstances normales, les fibres Aβ ne transmettent pas les informations nociceptives (Millan, 1999) mais peuvent devenir des nocicepteurs dans certaines conditions telles que, les douleurs neuropathiques (Nagi, et al., 2019).

Aγ : les fibres afférentes des récepteurs de l'étirement.

Aδ : sont des nocicepteurs mécaniques et thermiques.

Les fibres du groupe B sont modérément myélinisées et regroupent les fibres nerveuses préganglionnaires du système autonome (Guyton and Hall, 1986).

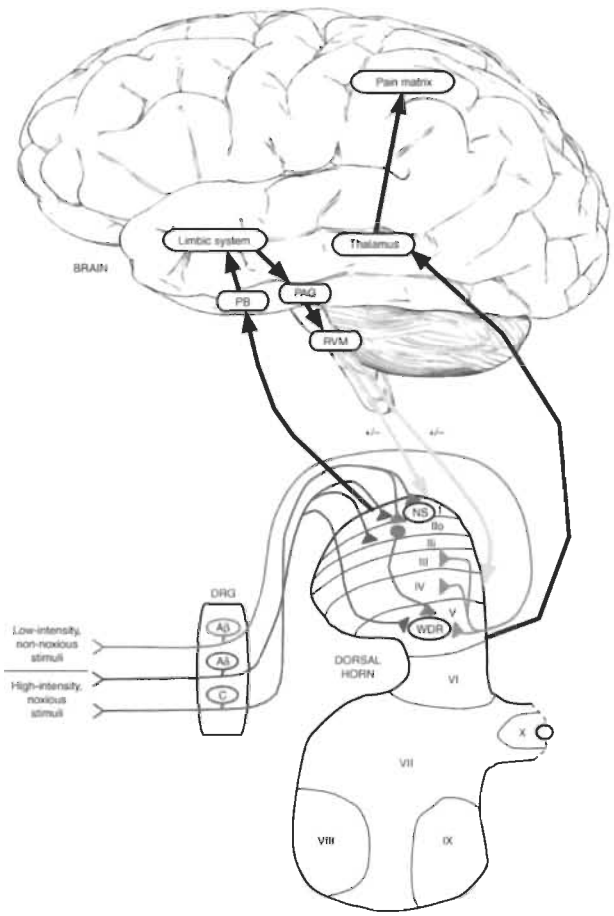
Finalement, les fibres du groupe C, non myélinisées avec un petit diamètre, conduisent l'information nociceptive thermique, chimique et mécanique (Guyton and Hall, 1986).

Parmi toutes ces fibres nerveuses, les fibres qui conduisent les informations nociceptives, en circonstances normales, sont donc les fibres Aδ et C.

Les afférences primaires des fibres nociceptives Aδ et C se projettent dans la corne dorsale de la moelle épinière principalement dans les couches I, II et avec un moindre degré dans les couches plus profondes (Light and Perl, 1979; Sugiura, et al., 1986) (Figure 1-1). La moelle épinière contient différents types de neurones qui font synapses avec les terminaisons des fibres afférentes primaires. Parmi ces neurones, les neurones spécifiques de nociception (NS) qui ont des connexions avec les fibres Aδ et C. Les NS sont localisés principalement dans les couches supérieures de la moelle épiniére et répondent exclusivement aux stimuli nociceptifs (Willis Jr and Coggeshall, 1991). Le deuxième type de neurones sont les neurones non nociceptifs ou à bas seuil localisés dans les couches III à V. Ils ont des connexions avec les fibres Aβ et sont principalement activés par des stimulations à bas seuil, non nociceptives (Willis Jr and Coggeshall, 1991) . Les neurones du 3ème type sont les neurones à large gamme dynamique (WDR) localisés principalement dans les couches IV et V. Ils reçoivent des inputs des fibres Aβ, Aδ et C et sont activés par différents types de stimulations allant des stimulations tactiles à bas seuil jusqu'aux stimulations nociceptives (Willis Jr and Coggeshall, 1991).

À partir de la moelle épinière, ces neurones se projettent vers les centres supérieurs du cerveau (Figure 1-1). Une partie des neurones nociceptifs se projette, via la voie spinothalamique, vers le thalamus, qui transmet à son tour les inputs nociceptifs vers différentes régions corticales constituant ce qu'on appelle "la matrice de la douleur" (Millan, 1999). Parmi ces régions le cortex préfrontal, les cortex somatosensoriels primaire et secondaire, le cortex cingulaire et le cortex insulaire (Almeida, et al., 2004; Tracey and Mantyh, 2007; D'Mello and Dickenson, 2008). Une autre cible majeure des fibres nociceptives est le noyau parabrachial

(PB). Les inputs des fibres Aδ et C se projettent, via la voie spino-parabrachiale, vers le PB et par la suite vers le noyau central de l'amygdale (CeA), le noyau du lit de la strie terminale (BNST), le noyau paraventriculaire du thalamus (PVN) ainsi que l'hypothalamus (Wiberg and Blomqvist, 1984; Gauriau and Bernard, 2002). Parmi les structures citées, cibles du PB, l'amygdale représente une structure particulièrement importante pour l'acheminement, le traitement et la modulation des informations nociceptives (Gauriau and Bernard, 2002; Veinante, et al., 2013).



Source : R. D'Mello, A.H. Dickenson, Spinal cord mechanisms of pain, Br J Anaesth, 101 (2008) 8-16

Figure 1-1 Les principales voies de la douleur

Les fibres afférentes primaires Aδ, Aβ et C transmettent les flux nociceptifs de la périphérie jusqu'à la moelle épinière. Les neurones spécifiques de nociception (NS) se trouvent majoritairement dans les couches supérieures (I et II) alors que la plupart des neurones à large gamme dynamique (WDR) se trouvent dans les couches profondes IV et V. Les neurones de la couche V se dirigent vers le thalamus qui envoie à son tour les inputs nociceptifs à différentes régions corticale qui forment "La matrice de la douleur". La majeure partie des neurones de la couche I se dirigent vers l'aire parabrachiale (PB) qui envoie des inputs à l'amygdale.

I.2.2.6 L'amygdale structure clé pour le traitement et la modulation de la douleur

L'amygdale est un complexe nucléaire en forme d'amande située dans le lobe temporal chez les mammifères et identifiée par Burdach au début du 19^{éme} siècle (Burdach, 1819-1822).

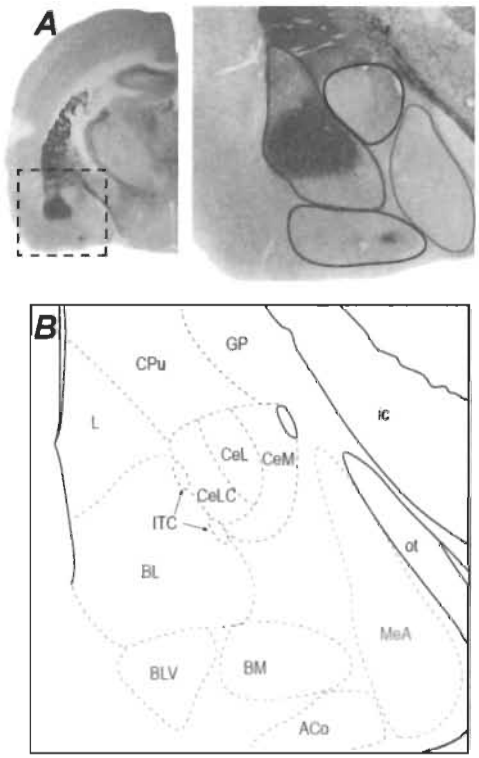
L'amygdale est une structure clé pour les émotions, l'attention ainsi que le traitement et la modulation de la douleur (Fanselow and LeDoux, 1999; Fanselow and Gale, 2003; Neugebauer, et al., 2004; Veinante, et al., 2013; Janak and Tye, 2015; Neugebauer, 2015).

L'importance de l'amygdale dans le traitement et la modulation de la douleur a été démontrée dans une panoplie d'études. Parmi les premières preuves du rôle indispensable de l'amygdale dans le traitement de la douleur, le cas célèbre du patient H.M (Hebben, et al., 1985). Le patient H.M a subi une résection bilatérale de l'amygdale, de l'hippocampe et du gyrus parahippocampique afin de corriger son épilepsie. Après cette chirurgie, il ne ressentait plus les stimuli thermiques même de forte intensité, perçus comme douloureux par d'autres sujets contrôles (Hebben, et al., 1985). Hebben (1985) a suggéré que cette absence de perception de la douleur, est probablement due à la résection de l'amygdale puisque toutes les autres structures impliquées dans le traitement de la douleur étaient intactes. Le rôle de l'amygdale dans le traitement de la douleur a été aussi démontré dans des études plus récentes. Chez l'humain et les modèles animaux de douleur, les études comportementales et physiologiques ont montré une plasticité fonctionnelle dans l'amygdale dans différentes conditions de douleur (Bernard, et al., 1992; Bornhovd, et al., 2002; Veinante, et al., 2013). Dans l'une des études réalisées chez la souris, il a été montré que le blocage pharmacologique des ERK (Extracellular signal-regulated kinases) dans l'amygdale réduit l'hypersensibilité périphérique mécanique alors

que l'administration des ERK induit une hypersensibilité périphérique aux stimuli tactiles (Carrasquillo and Gereau, 2007).

L'amygdale est composée de différents noyaux et régions (Figure 1-2). Le noyau central de l'amygdale (CeA) contient la plupart des noyaux qui répondent aux stimuli nociceptifs. L'amygdale reçoit les informations nociceptives du thalamus et de différentes régions corticales vers son noyau basolatéral (BLA) qui les transmet à son tour au noyau central (McDonald, et al., 1999). Le CeA reçoit également des informations nociceptives directes du PB (Gauriau and Bernard, 2002) (Figure 1-3) et participe dans la modulation descendante de la douleur par le biais de ses denses connexions avec le PAG (Pavlovic and Bodnar, 1998) et le PB (De Olmos, et al., 2004; Sarhan, et al., 2005).

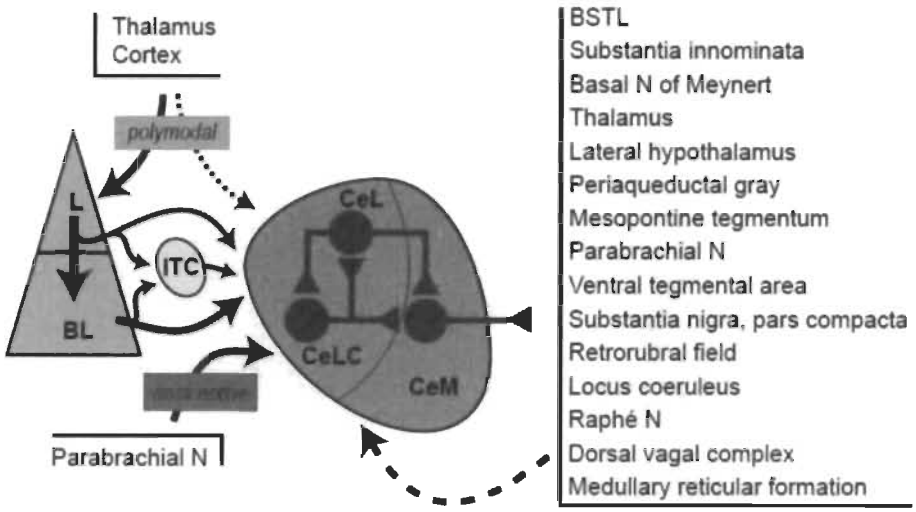
Les études chez l'animal ont mis en évidence le rôle important du CeA dans le traitement et la modulation de la douleur. Chez les rats anesthésiés, les stimulations mécaniques ou thermiques (Bernard, et al., 1992; Neugebauer and Li, 2002), articulaires, musculaires (Neugebauer and Li, 2003; Neugebauer, et al., 2003), et viscérales (Nakagawa, et al., 2003; Suwanprathes, et al., 2003) induisent des changements fonctionnels dans le CeA. D'autres études animales ont également montré une augmentation de l'activité du CeA dans le cas de douleurs chroniques inflammatoires (Carrasquillo and Gereau, 2007), viscérales (Greenwood-Van Meerveld, et al., 2001; Nishii, et al., 2007) et neuropathiques (Ikeda, et al., 2007; Cooper, et al., 2018). En outre, des études réalisées chez l'animal ont montré que le blocage des récepteurs glutamergiques mGluR1 inhibe la vocalisation liée à la douleur ainsi que les réflexes spinaux chez les modèles de souris d'arthrite (Han and Neugebauer, 2005). De plus, l'activation des ERK pro-nociceptifs dans le CeA produit une hypersensibilité mécanique chez le modèle de souris d'arthrite (Fu, et al., 2008).



Source: P. Veinante, I. Yalcin, M. Barrot, The amygdala between sensation and affect: a role in pain, Journal of Molecular Psychiatry, 1 (2013) 9. (Veinante, et al., 2013)

Figure 1-2 Organisation anatomique de l'amygdale

A : Section frontale de cerveau de rat. Les 4 sous-groupes de l'amygdale délimités sont : basolatéral (rouge), central (bleu), superficiel (violet) et médial (vert) B : Les différents sous-noyaux des sous-groupes de l'amygdale. Le groupe basolatéral est composé de 4 sous-noyaux : latéral (L), basolatéral (BL), basolatéral ventral (BLV) et basolatéral médial (BM). Le groupe central (bleu) est composé de 3 sous-noyaux : latéral (CeL), capsulaire (CeLC) et médial (CeM). Le groupe médial et le groupe superficiel sont représentés par le noyau médial (MeA) et le noyau cortical antérieur (Aco)



Source: P. Veinante, I. Yalcin, M. Barrot, The amygdala between sensation and affect: a role in pain, Journal of Molecular Psychiatry, 1 (2013) 9. (Veinante, et al., 2013)

Figure 1-3 Principales connexions du noyau central impliquées dans le traitement de la douleur

Le CeA reçoit des informations polymodales du cortex cérébral et du thalamus à travers le noyau basolatéral (BL) et latéral (L) ainsi que des informations nociceptives directes à travers le noyau parabrachial (PB).

L'amygdale et son noyau central jouent donc un rôle très important dans l'hyperalgésie (Fu, et al., 2008; Goncalves, et al., 2008; Han, et al., 2010; Cheng, et al., 2011; Rouwette, et al., 2012) et la douleur spontanée (Rouwette, et al., 2012; Arimura, et al., 2019) dans le cas de douleurs chroniques. On pourrait donc penser que ce complexe amygdalien jouerait un rôle critique dans l'hypersensibilité à la douleur observée chez les aveugles congénitaux.

Dans la deuxième étude de cette thèse, la sensibilité à la douleur chez les souris anophtalmes a été examinée. Afin d'élucider les mécanismes sous-jacents à l'hyperalgésie observée chez les souris aveugles, nous avons investigué, par la suite, la plasticité fonctionnelle et structurelle dans l'amygdale.

Objectif 2: Évaluer la sensibilité à la douleur chez la souris aveugle.

Objectif 3: Investiguer la plasticité fonctionnelle et structurelle induite par la stimulation douloureuse dans l'amygdale et son noyau central.

Hypothèse 2: Une hypersensibilité à la douleur aiguë serait observée chez les souris aveugles.

Hypothèse 3: Des changements structurels et fonctionnels seraient observés au niveau de l'amygdale et de son noyau central.

I.2.3 Impacts structurels et fonctionnels de la cécité

Les adaptations comportementales suite à la perte de vision précoce discutées plus haut, sont étroitement liées à différentes modifications cérébrales anatomiques et fonctionnelles mises en évidence dans plusieurs études réalisées chez les aveugles. Dans cette partie, ces formes de plasticité cérébrale observées chez les aveugles seront discutées.

I.2.3.1 Définition de la plasticité cérébrale

La plasticité cérébrale est la capacité du cerveau à changer et à s'adapter. Il peut y avoir des modifications de volume de matière grise et/ou matière blanche de certaines structures ainsi que des modifications dans les connexions sous-corticales et cortico-corticales (Bennett, et al., 1964). Suite à une lésion touchant un système sensoriel donné, on distingue deux types de plasticité :

La *plasticité intramodale* qui concerne les modifications neuroanatomiques ayant lieu au sein d'une modalité sensorielle donnée (changements volumétriques, fonctionnels, synaptiques...) (Voss and Zatorre, 2012b; Zatorre, et al., 2012).

La *plasticité intermodale* qui concerne la réorganisation du réseau neuronal conduisant à l'intégration d'une ou plusieurs nouvelles modalités sensorielles dans une aire sensorielle donnée. La plasticité intermodale a souvent, mais pas toujours, lieu suite à une perte sensorielle précoce. Les modifications incluent une formation de nouvelles connexions cortico-corticales et une altération des connexions existantes (Bavelier and Neville, 2002; Voss and Zatorre, 2012b).

I.2.3.2 La plasticité cérébrale suite à la privation visuelle

L'étude des impacts cérébraux structurels et fonctionnels de la privation visuelle a commencé avec les travaux de Hess en 1958. Hess a étudié les effets neuroanatomiques de l'énucléation chez le cochon d'Inde au stade fœtal. Il a montré que suite à l'énucléation monoculaire ou binoculaire, des changements structurels dans les voies visuelles ont lieu. Il a mis en évidence une dégénérescence du nerf optique et du chiasma optique ainsi qu'une réduction de la taille des collicules supérieurs et du corps géniculé latéral du thalamus (Hess, 1958). Un peu plus tard, au début des années 60, David H. Hubel et Torsen N. Wiesel pionniers de la plasticité neuronale et corticale, ont étudié l'effet de la privation monoculaire et binoculaire chez les primates et chez le chat. Leurs travaux leur ont valu le prix Nobel en médecine en 1981. Les deux chercheurs ont étudié les conséquences de la privation visuelle monoculaire pendant une période de 2 à 3 mois chez des chatons après la naissance. Ils ont constaté un transfert des réponses dans le cortex visuel primaire de l'œil suturé vers l'œil intact (98% des neurones de l'œil suturé devenaient non sensibles aux stimuli) (Wiesel and Hubel, 1963). Par la suite, ils ont montré que suite à la privation binoculaire dès la naissance, plus que la moitié des neurones étaient non sélectifs et répondaient aux stimuli des deux yeux (Wiesel and Hubel, 1965). Ils ont par la suite essayé l'occlusion alternée des deux yeux et ont constaté que 91% des neurones répondaient soit à l'un ou l'autre œil, mais pas aux deux yeux ensemble (Wiesel and Hubel, 1965). Ils ont conclu que la perte des réponses neuronales de l'œil suturé était le résultat de processus compétitifs avec l'œil intact. Ils ont par la suite, testé la privation monoculaire chez les chatons à différents âges et différentes durées de privation afin de voir si les réponses physiologiques résultant de la privation visuelle étaient gouvernées par une période de développement critique. Ils ont démontré qu'il existe une période de susceptibilité qui commence à la naissance et qui dure environ 3 mois. Une privation monoculaire pendant les 3

premiers mois suivant la naissance, même de courtes durées (3-4 jours) résulte en un déclin permanent et irréversible dans la proportion des cellules qui réagissent à l'œil suturé, tandis qu'une privation monoculaire même de longue durée chez un chat adulte avait peu ou pas d'impacts physiologiques ou neuroanatomiques (Hubel and Wiesel, 1970). Ils étaient les premiers à faire cette observation de période critique de plasticité cérébrale suite à une perte sensorielle. Différentes questions se sont posées par la suite : qu'est-ce qu'on observerait au niveau des voies visuelles et au niveau cérébral en l'absence de tout input visuel binoculaire (cas de la cécité totale)? Est-ce qu'il y a des processus compétitifs entre le cortex occipital et les autres cortex? Plusieurs investigations de différents chercheurs ont suivi afin de répondre à ces questions et élucider la nature de la plasticité cérébrale liée à la perte visuelle ainsi que ses conséquences fonctionnelles et comportementales.

I.2.3.3 Période critique de plasticité : Aveugles précoces vs aveugles tardifs

Comme démontré par Hubel et Wiesel, il existe une période critique de plasticité pendant laquelle les systèmes sensoriels sont sensibles aux stimuli externes et se réorganisent selon les expériences sensorielles vécues. Ces expériences modifient les connexions et les circuits neuronaux au fur et à mesure jusqu'à la maturation, correspondant à la fin de la période critique de plasticité qui diffère d'un système à l'autre. Au-delà de cette période, peu de changements neuronaux peuvent survenir et le cerveau devient moins plastique (Sale, et al., 2010). L'âge de la perte sensorielle est donc extrêmement important et déterminant du degré de l'adaptation comportementale et cérébrale.

L'une des premières études de neuroimagerie examinant l'activité métabolique du cortex occipital chez les humains aveugles a comparé des aveugles précoces et tardifs à des contrôles voyants. Cette étude a montré que l'activité fonctionnelle du cortex occipital chez les aveugles tardifs était différente de celle des aveugles précoces. En effet, les aveugles précoces ont montré une activité métabolique au repos plus élevée dans le cortex occipital comparés aux individus contrôles voyants tandis que les aveugles tardifs ont montré une activité métabolique réduite comparée aux contrôles (Wanet-Defalque, et al., 1988; Veraart, et al., 1990). Cette étude a démontré que l'âge de perte de vision est un facteur déterminant des modifications pouvant avoir lieu au niveau du cortex occipital. D'autres études ont appuyé cette notion. Des études de

neuroimagerie où les participants devaient accomplir des tâches non visuelles ont montré un recrutement de cortex occipital chez les aveugles précoces témoignant d'une plasticité intermodale. Cette activité au niveau du cortex occipital était absente chez les aveugles tardifs (Cohen, et al., 1999; Sadato, et al., 2002). D'autres études ont contredit ces conclusions et ont pu mettre en évidence des modifications dans le cortex occipital au-delà de la période critique. Une activation du cortex occipital en réponse à une variété de tâches auditives et tactiles a été observée chez les aveugles tardifs (Kujala, et al., 1997; Burton, et al., 2002; Burton, et al., 2004; Burton and McLaren, 2006). Cependant, dans la plupart de ces études, il n'y avait de changements comportementaux associés à ces modifications cérébrales chez les aveugles tardifs contrairement aux aveugles précoces. Chez les animaux, la seule étude qui a comparé les aveugles précoces aux aveugles tardifs a été réalisée par Chabot et ses collaborateurs (Chabot, et al., 2007) dans notre laboratoire. Dans cette étude, en utilisant des techniques d'immunohistochimie, on a mis en évidence des différences dans l'activation du cortex occipital suite à des stimuli auditifs entre les souris aveugles tardives (énuclées après la naissance) et des souris aveugles congénitales. En effet, chez les souris aveugles congénitales une activation a été observée dans le cortex visuel primaire (V1), le cortex visuel secondaire (V2) et le noyau géniculé dorslatéral (DLG) du thalamus, relais important pour le traitement de l'information visuelle, tandis que seulement V2 et à peine V1 étaient activés chez les aveugles tardifs (Chabot, et al., 2007). La perte de vision a donc des impacts variables dépendamment de l'âge de cécité. Dans la partie suivante, les différents impacts cérébraux structurels et fonctionnels de la perte de vision seront abordés.

I.2.3.4 Impacts fonctionnels de la cécité

Plasticité intermodale

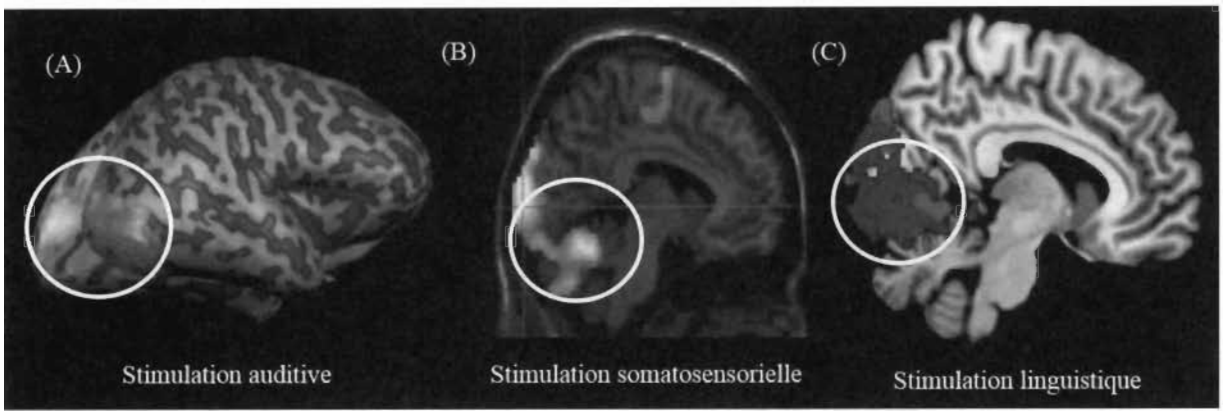
Le système visuel est topographiquement organisé en zones anatomiquement distinctes, chacune étant dédiée à un ensemble spécifique de fonctions. Le cortex occipital est par exemple spécialisé dans le traitement de l'information visuelle. Mais le cortex visuel conserve-t-il cette organisation fonctionnelle spécialisée quand il est privé des inputs visuels?

La perte d'un sens donné résulte en une invasion de la structure cérébrale déprivée par les inputs des autres modalités sensorielles ce qui démontre la capacité impressionnante du

cerveau à s'adapter et se réorganiser (Ptito and Desgent, 2006; Merabet and Pascual-Leone, 2010; Ricciardi and Pietrini, 2011). Ce phénomène de plasticité a été abordé pour la première fois en 1977 par Rebillard et al. (1977) qui ont rapporté que le cortex auditif chez les chats sourds congénitaux est activé par des stimuli visuels. Bronchti et al. (1989; 2002) ont montré le même phénomène de plasticité en démontrant que des stimuli auditifs activent le noyau géniculé dorsolatéral et le cortex visuel chez le rat-taupe microphtalmique (*Spalax ehrenbergi*). Suite à une privation visuelle, les cellules du cortex visuel primaire deviennent donc sensibles aux stimuli auditifs et somatosensoriels.

Par exemple, chez le rat énucléé à la naissance, les stimuli somatosensoriels (stimulation de la base des vibrisses) activent le cortex visuel primaire (Toldi, et al., 1994).

L'avènement récent des outils d'imagerie cérébrale a permis de mieux comprendre et d'investiguer davantage les modifications cérébrales chez l'aveugle. Kupers et al. (2009) ont montré que l'épaisseur du cortex visuel ainsi que son activité métabolique sont plus importantes chez les aveugles congénitaux comparés aux voyants. En utilisant des techniques d'imagerie cérébrale fonctionnelle, Uhl et al. (1991; 1993) étaient parmi les premiers à montrer une activation cérébrale dans le cortex occipital induite par des stimuli tactiles chez les humains aveugles précoces. Par la suite, plusieurs autres études, ont montré que le cortex occipital chez les individus aveugles précoces est impliqué dans le traitement de différents processus cognitifs non visuels (Roder, et al., 1999; Amedi, et al., 2003; Amedi, et al., 2004; Kupers and Ptito, 2004; Kupers, et al., 2007; Kupers, et al., 2010; Cuevas, et al., 2011; Kolarik, et al., 2014) (Figure 1-4).



Source : modifié de A.K. Singh, F. Phillips, L.B. Merabet, P. Sinha, Why Does the Cortex Reorganize after Sensory Loss?, Trends in cognitive sciences, 22 (2018) 569-582. (Singh, et al., 2018)3

Figure 1-4 Plasticité intermodale : Exemples d'activation du cortex occipital lors de traitement de tâches non visuelles

Réponse du cortex occipital (cercle blanc) à des stimuli non visuels
(A) Les aires corticales occipitales plus activées chez les aveugles précoces que chez les voyants lors de stimuli auditifs (Renier, et al., 2010) (B) Activation du cortex occipital pendant la lecture Braille comparée à l'activation au repos chez des aveugles précoces (Gizewski, et al., 2003) (C) Une activité plus importante dans le cortex occipital chez les aveugles comparés aux voyants suite à une stimulation auditive linguistique (Bedny, et al., 2011)

Rôle fonctionnel du cortex occipital chez l'aveugle

Les études montrant l'activation du cortex occipital chez les aveugles par des tâches non visuelles ne permettent pas d'établir une causalité entre cette activation et les performances comportementales.

Afin de tester le rôle fonctionnel du cortex occipital chez l'aveugle précoce et d'établir le lien avec le comportement, des techniques de stimulation magnétique transcrânienne (TMS) ont été employées. La TMS est une technique qui permet, selon la fréquence utilisée, d'activer ou d'inhiber une région corticale donnée. Des études ont montré qu'une inhibition du cortex occipital par TMS chez des aveugles précoces résulte en une diminution de leur perception tactile et une augmentation du nombre d'erreurs pendant une tâche de lecture Braille (Cohen, et al., 1997; Kupers, et al., 2007). Une autre étude a montré que l'application de TMS au niveau

du cortex occipital diminue les performances des aveugles précoces dans une tâche sémantique (Amedi, et al., 2004). Ces études prouvent que les modifications fonctionnelles observées au niveau du cortex occipital chez les aveugles influent leurs performances dans les tâches non visuelles.

La plasticité cérébrale chez les aveugles est plus étendue et induit des modifications cérébrales structurelles.

I.2.3.5 Impacts structurels de la cécité

Plasticité intramodale

En plus des changements fonctionnels, des changements neuroanatomiques sous-corticaux et corticaux sont observés suite à la privation visuelle dans le cortex occipital et ailleurs dans le cerveau. Les études animales montrent que la cécité précoce résulte en une atrophie des voies visuelles de la rétine jusqu'au cortex visuel (Movshon and Van Sluyters, 1981; Kahn and Krubitzer, 2002; Karlen, et al., 2006). Chez les humains aveugles congénitaux et anophtalmes, des techniques d'IRM et de tractographie ont été employées afin d'étudier les altérations de la matière grise et de la matière blanche *in vivo*. Les analyses montrent une atrophie de la matière grise de toutes les voies visuelles, incluant le DLG, le pulvinar postérieur et les aires visuelles striées et extra striées (Noppeney, et al., 2005; Pan, et al., 2007; Ptito, et al., 2008) ainsi qu'une réduction volumétrique du cortex moteur et du cortex cingulaire postérieur (Leporé, et al., 2010). Une augmentation de volume a été observée dans d'autres régions. Par exemple, une augmentation du volume de la partie supérieure de l'hippocampe, une région impliquée dans les tâches de navigation, a été mise en évidence chez l'aveugle précoce (Fortin, et al., 2008). Des études ont également montré une augmentation de volume du cortex auditif primaire (Elbert, et al., 2002) et des bulbes olfactifs (Rombaux, et al., 2010). Des modifications de la connectivité cortico-corticale sont également observées. Ptito et al. (2008) ont montré que la cécité précoce résulte en une réduction du volume de la matière blanche dans les tractus reliant des régions dans le cortex temporal et le cortex occipital. La même équipe a observé une augmentation du volume de la matière blanche dans les tractus reliant le cortex préfrontal et le cortex occipital latéral (Ptito, et al., 2008). Chez les animaux aveugles, les études ont montré une atrophie des cortex visuels et collicules supérieurs (Lund and Lund, 1971; Rhoades, 1980;

Rhoades, et al., 1984) ainsi qu'une augmentation du volume du cortex auditif primaire (Gyllensten, et al., 1966).

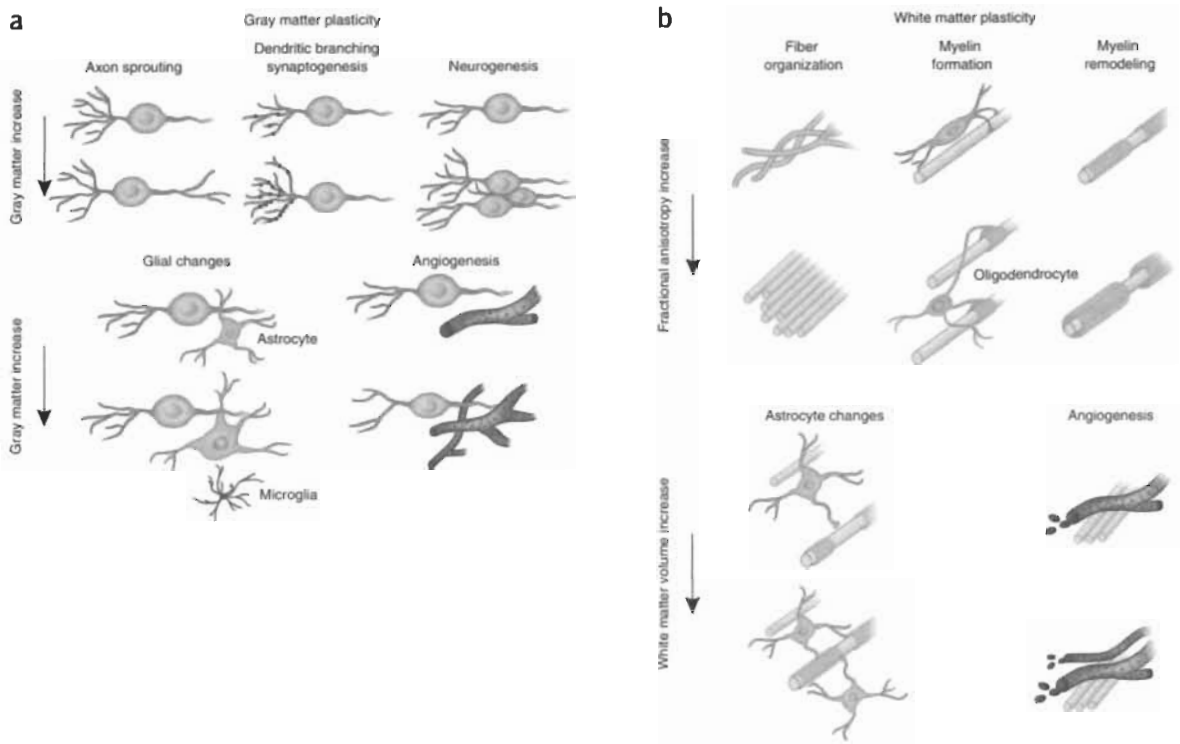
Les modifications neuroanatomiques observées chez les aveugles précoces ont une pertinence fonctionnelle et sont étroitement liées aux modifications comportementales mises en évidence chez ces derniers.

Mécanismes sous-jacents à la plasticité intramodale

Plusieurs facteurs peuvent induire des changements structurels cérébraux. L'entraînement et/ou l'apprentissage de nouvelles compétences, par exemple, peuvent résulter en une augmentation du volume de certaines structures cérébrales. Il a été montré, par exemple, que les chauffeurs de taxi ont une hypertrophie de l'hippocampe, zone impliquée dans la navigation spatiale (Maguire, et al., 2000). Des études ont également montré une hypertrophie des cortex auditifs chez les musiciens (Schneider, et al., 2002; Bermudez, et al., 2009). La taille des structures corticales dépend donc de l'expérience. Dans le cas de la cécité, l'utilisation supranormale des structures non visuelles comme mécanisme adaptatif peut résulter en une augmentation du volume de ces dernières. Mais comment se produisent ces changements structurels? Quels sont les mécanismes cellulaires et moléculaires sous-jacents?

Les changements structuraux peuvent être catégorisés en changements neuronaux dans la matière grise et la matière blanche ainsi que des changements extraneuronaux (Zatorre, et al., 2012) qui, tous, peuvent influencer les signaux IRM (Figure 1-5). Les changements neuronaux dans la matière grise incluent la neurogenèse, la synaptogenèse et le changement de la morphologie neuronale (Figure 1-5). Dans la matière blanche, les changements incluent le changement du nombre des axones, leurs diamètres, leur arborisation, leurs trajectoires et leur myélinisation (Figure 1-5). Les changements extraneuronaux incluent l'augmentation de la taille et/ou le nombre des cellules gliales ainsi que l'angiogenèse (Zatorre, et al., 2012) (Figure 1-5). Ces changements contribuent à l'augmentation du volume et/ou de l'épaisseur corticale. Mais une atrophie volumétrique corticale peut également être observée dans le cas contraire où il y a plutôt une désuétude d'une région en particulier causée par une perte sensorielle. Dans le cas de la cécité, par exemple, la désuétude conduit à une atrophie du cortex visuel (Noppeney, et al., 2005; Ptito, et al., 2008). Cette atrophie peut être causée par une réduction de la densité

synaptique, réduction du nombre des axones, de leurs arborisations ou de leur myélinisation (Globus and Scheibel, 1966). Puisque l'expérience est le principal stimulant de la maturation neuronale, un entraînement peut conduire à une expansion neuronale alors qu'une privation peut induire une absence de maturation (cas de la cécité).



Source : R.J. Zatorre, R.D. Fields, H. Johansen-Berg. Plasticity in gray and white: neuroimaging changes in brain structure during learning. Nat Neurosci. 15 (2012) 528-53

Figure 1-5 Mécanismes cellulaires conduisant à l'hypertrophie

a) Mécanismes cellulaires sous-jacents de l'expansion de la matière grise incluant neurogenèse, la synaptogenèse, croissance axonale et l'angiogenèse b) Changements dans la matière blanche incluant l'altération de l'organisation des fibres, myélinisation, le nombre des axones, leurs diamètres, leurs trajectoires, changements des astrocytes et angiogenèse

Afin d'étudier davantage les impacts de la cécité précoce sur la plasticité cérébrale, dans la 3éme étude de cette thèse, nous avons examiné les changements cérébraux morphométriques chez le modèle de souris aveugles ZRDBA.

Objectif 4: Étudier l'impact de la cécité précoce sur la plasticité cérébrale dans les structures visuelles et non visuelles chez le modèle de souris aveugle ZRDBA.

Hypothèse 4: Une atrophie serait observée dans les structures visuelles incluant les cortex visuels primaire et secondaire ainsi que les collicules supérieurs chez les souris aveugles. Une augmentation du volume serait observée au niveau de l'amygdale, du cortex auditif ainsi que des structures olfactives.

I.3 Considérations méthodologiques

I.3.1 La souche ZRDBA

La souche ZRDBA est issue du croisement de la souche anophtalme ZRDCT et de la souche voyante DBA-6. La souche ZRDCT a été décrite pour la première fois en 1941 par Chase et Chase (1941). Plus que 90 % des souris de cette souche homozygotes pour un déterminant génétique récessif naissent anophtalmes (Chase, 1944). Ces souris ont des orbites, mais elles n'ont ni yeux ni nerfs optiques (Chase and Chase, 1941). En 2001, Tucker et al. ont fourni la description génétique de la souche. Le phénotype "absence des yeux" est dû à une mutation sur le locus récessif (ey1) du chromosome 18 codant pour le gène Rx/Rax. Ce gène est exprimé dans la partie rostrale de l'embryon et il est impliqué dans le développement de la rétine, de l'hypothalamus et de la glande pinéale (Tucker, et al., 2001). En plus de l'absence des yeux et des nerfs optiques, cette mutation induit l'absence des afférences rétiniennes à l'hypothalamus (Tucker, et al., 2001), développement de fibres auditives dans le DLG (Piche, et al., 2004; Chabot, et al., 2007) ainsi qu'une asymétrie du noyau supra chiasmatique (Laemle and Rusa, 1992). Le noyau supra chiasmatique est responsable de la régulation du rythme circadien chez les rongeurs (Inouye and Kawamura, 1982). Cependant, il a été démontré que malgré cette asymétrie et malgré l'absence des afférences rétiniennes, le rythme circadien chez cette souche aveugle est régulé par des stimuli non-photiques (Laemle and Ottenweller, 1999). Aucune autre anomalie cérébrale majeure n'a été décrite chez la souche de souris ZRDCT.

La souche ZRDCT a été utilisée dans plusieurs études afin d'étudier les impacts de la cécité sur la plasticité cérébrale (Rhoades, et al., 1984; Laemle, et al., 2006; Charbonneau, et al., 2012; Masse, et al., 2014). Cependant, dans ces études, les souris ZRDCT ont souvent été comparées aux souris provenant d'autres souches comme la souche C57Bl/6 pouvant présenter des différences comportementales et cérébrales, autres que celles induites par la cécité, avec la souche ZRDCT. D'où la pertinence de la souche nouvellement développée ici, ZRDBA, qui comporte des souris voyantes et des souris aveugles dans les mêmes portées et qui permet d'étudier les impacts de la cécité sur le développement comportemental et cérébral sans le biais des différences inter-souches.

Afin d'obtenir une souche ZRDBA stable, des croisements ont été faits sur cinq générations. Le premier croisement a été réalisé entre une souris DBA-6 et une souris ZRDCT afin d'obtenir une souris hybride, hétérozygote F1. Quatre rétrocroisements ont suivi, chaque fois entre une hétérozygote pour la mutation, donc voyante, et une ZRDCT homozygote (aveugle) : F1 X ZRDCT, F2 X ZRDCT, F3 X ZRDCT, F4 X ZRDCT. À chaque génération, les souris qui étaient utilisées pour le rétrocroisement étaient testées génétiquement de Charles Rivers pour s'assurer de leur proximité génétique croissante avec la souche ZRDCT. Après les 5 croisements, Charles Rivers nous a certifié la stabilité de la nouvelle souche que nous avons nommée ZRDBA. Nous avons donc continué par des croisements internes (inbreeding) sœur-frère, toujours en croisant un hétérozygote avec un homozygote, ce qui nous assure d'obtenir 50% d'hétérozygotes et 50% d'homozygotes. Nous n'observons que très peu de souris présentant des anomalies (yeux blancs, un seul œil...). Après 10 générations, toujours selon les conseils des spécialistes de Charles Rivers, un rétrocroisement a été réalisé avec une ZRDCT sur deux générations pour réduire les dérives génétiques possibles dans les colonies de souris.

Dans la souche ZRDBA, donc, la moitié des souris naissent anophtalmes alors que l'autre moitié des souris naissent voyantes, et ceci dans une même portée (voir figure 1-6) ce qui permet d'étudier l'impact de la cécité précoce sur les fonctions sensorielles et la plasticité cérébrale dans une même souche. Pour autant que nous sachions, c'est la seule souche de ce type. Les souris aveugles et les souris voyantes sont logées dans des cages enrichies mixtes dans des installations locales avec un cycle lumière / obscurité de 14 h / 10 h.

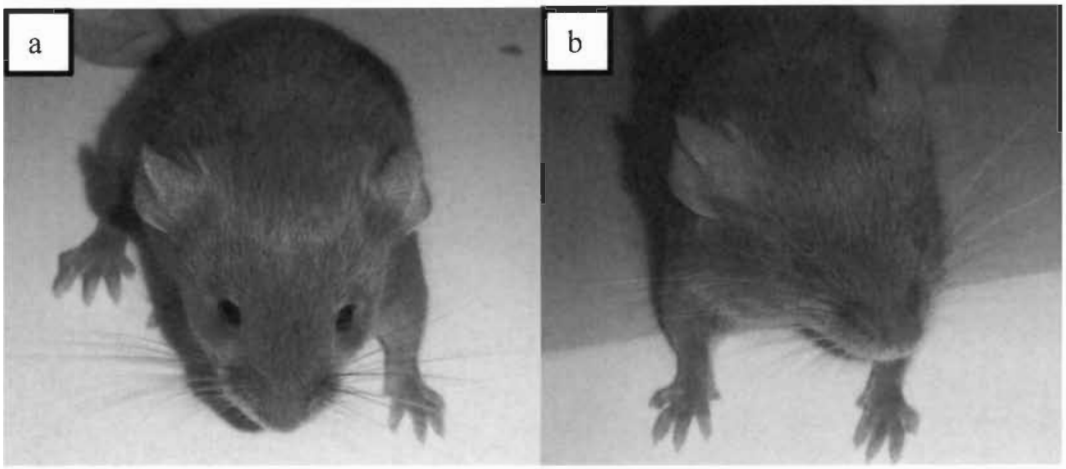


Figure 1-6 Photos des souris de la souche ZRDBA

a : Souris voyante de la souche ZRDBA, b : Souris anophtalme de la souche ZRDBA

I.3.2 Les tests comportementaux

I.3.2.1 Les tests d'olfaction

Dans la première étude de cette thèse, les performances olfactives des souris aveugles et voyantes de la souche ZRDBA ont été testées à l'aide de trois tests olfactifs. Ces trois tests accessibles et faciles à réaliser sont des tests standardisés qui ont été employés dans plusieurs études (Witt, et al., 2009; Canugovi, et al., 2015) . Ils permettent de tester la fonction olfactive spécifiquement et de la comparer entre les deux groupes de souris (voir détails des tests dans le chapitre II).

Pour les trois tests, les souris ont été habituées à la cage de test 30min/jour pendant 5 jours. Tous les tests ont été filmés et analysés par le logiciel Ethovision. Le premier test est celui de recherche de nourriture qui permet de mesurer la latence avant de retrouver une croquette cachée en dessous de la litière de la cage (voir figure 1-7a). Les souris sont privées de nourriture pendant 18h avant le test. Dans ce test, les souris se basent uniquement sur leur odorat afin de retrouver la nourriture dans la cage. Le deuxième test est le test de seuil olfactif qui permet de déterminer la sensibilité olfactive des animaux testés en déterminant la concentration de l'odeur la plus basse qu'ils peuvent détecter. Dans ce test, les animaux sont exposés à une série de dilutions des

odeurs testées (rose et cannelle) et le temps passé sur chaque dilution est calculé et est comparé avec les autres dilutions et entre les groupes de souris (voir figure 1-7b). Le troisième test est le test de performance olfactive qui permet d'évaluer la capacité des animaux à différencier les odeurs et à distinguer une odeur attractive d'une odeur aversive. Dans ce test, les animaux ont été exposés à trois types d'odeurs : vanille (attractive), beurre de cacahuète (attractive) et 2-Methylbutyric acid (2MB) (aversive) (Witt, et al., 2009; Canugovi, et al., 2015). Le papier filtre imbibé par l'odeur était à chaque fois présenté avec un autre papier filtre imbibé par seulement de l'eau (voir figure 1-7c). Le temps passé sur chaque odeur versus le temps passé sur l'eau a été mesuré et comparé entre les souris aveugles et les souris voyantes.

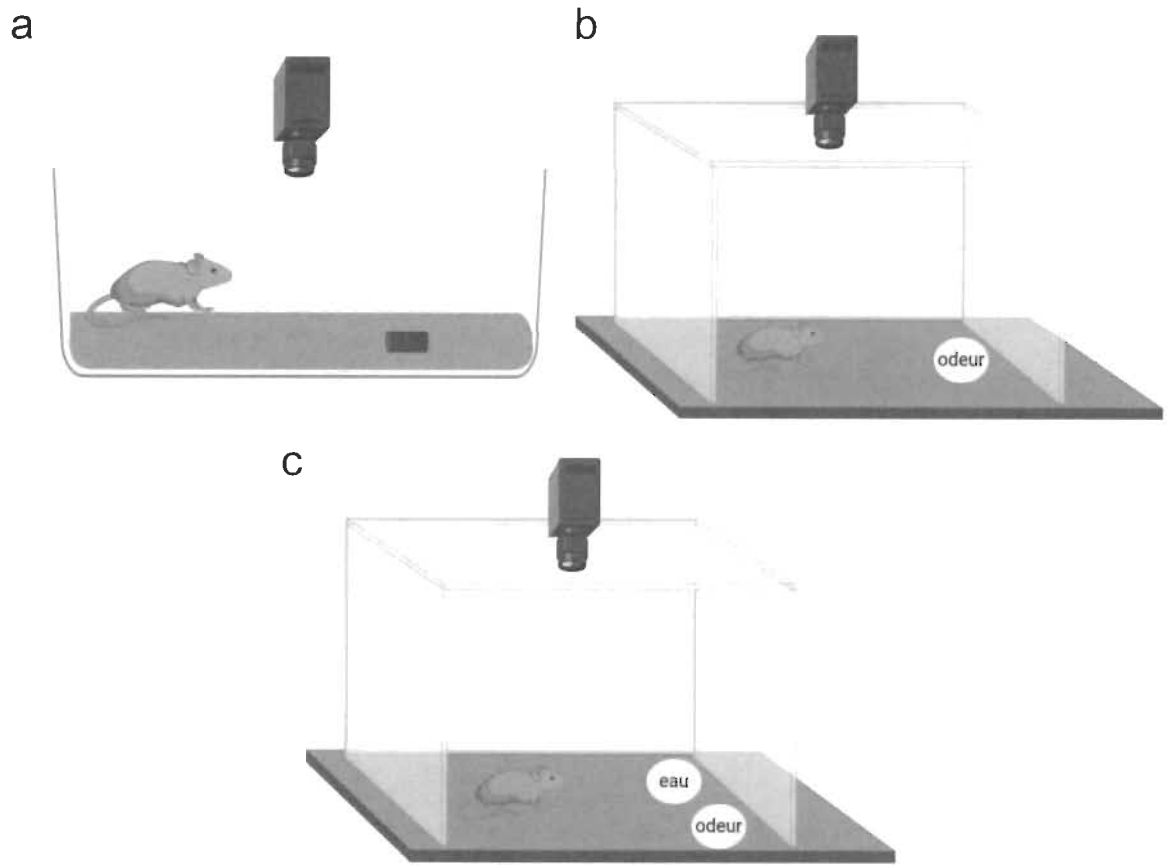


Figure 1-7 Tests olfactifs

a : test de recherche de nourriture, b : test de seuil olfactif, c : test de performance olfactive

I.3.2.2 Les tests de douleur

Dans la deuxième étude de cette thèse la sensibilité à la douleur chez les souris aveugles a été évaluée par le biais de quatre tests: 1- Le test de Von Frey qui permet de tester la sensibilité mécanique. Dans ce test, des filaments de von Frey de diamètres ascendants sont appliqués sur la face dorsale de la patte jusqu'à l'observation d'une réponse positive (léchage, retrait ou remuement de la patte). Une valeur de calibration correspondant au logarithme décimal de la force (en mg) requise pour plier le filament est assignée à chaque filament. Le filament qui induit une réponse positive est considéré comme étant le seuil mécanique de l'animal (voir figure 1-8a). Les seuils mécaniques sont comparés entre les différents groupes d'animaux. 2- Le test d'acétone qui permet de tester la sensibilité au froid. Dans ce test une goutte d'acétone pure est appliquée sur la patte de l'animal et la latence avant l'observation d'une réponse positive (léchage, retrait ou remuement de la patte) est calculée et comparée entre les animaux (voir figure 1-8b). 3- Le test de water tail-flick qui permet de tester la sensibilité à la chaleur (voir figure 1-8c). Ce test consiste à plonger les deux tiers de la partie distale de la queue de l'animal dans de l'eau chaude maintenue à 48° C et la latence jusqu'au retirement de la queue est calculée et comparée entre les groupes d'animaux. 4- Le test de formaline qui permet de tester la sensibilité à un stimulus chimique (voir figure 1-9d). Dans ce test, l'animal reçoit une injection d'une solution de 5% formaline dans la face dorsale de la patte et le comportement de douleur (léchage de la patte) est observé et quantifié pendant 1h suivant l'injection (voir détails des tests dans le chapitre II). Ce test servira également à mesurer l'activation cérébrale par la mesure de la protéine c-Fos induite par la douleur suite à l'injection de la formaline (voir 1.3.4.2 1.3.4. la protéine c-Fos). Chez l'humain aveugle, seule la sensibilité thermique a été testée. Dans la présente étude, les tests réalisés permettent d'examiner la sensibilité à la douleur conséquente de la cécité dans les autres modalités sensorielles.

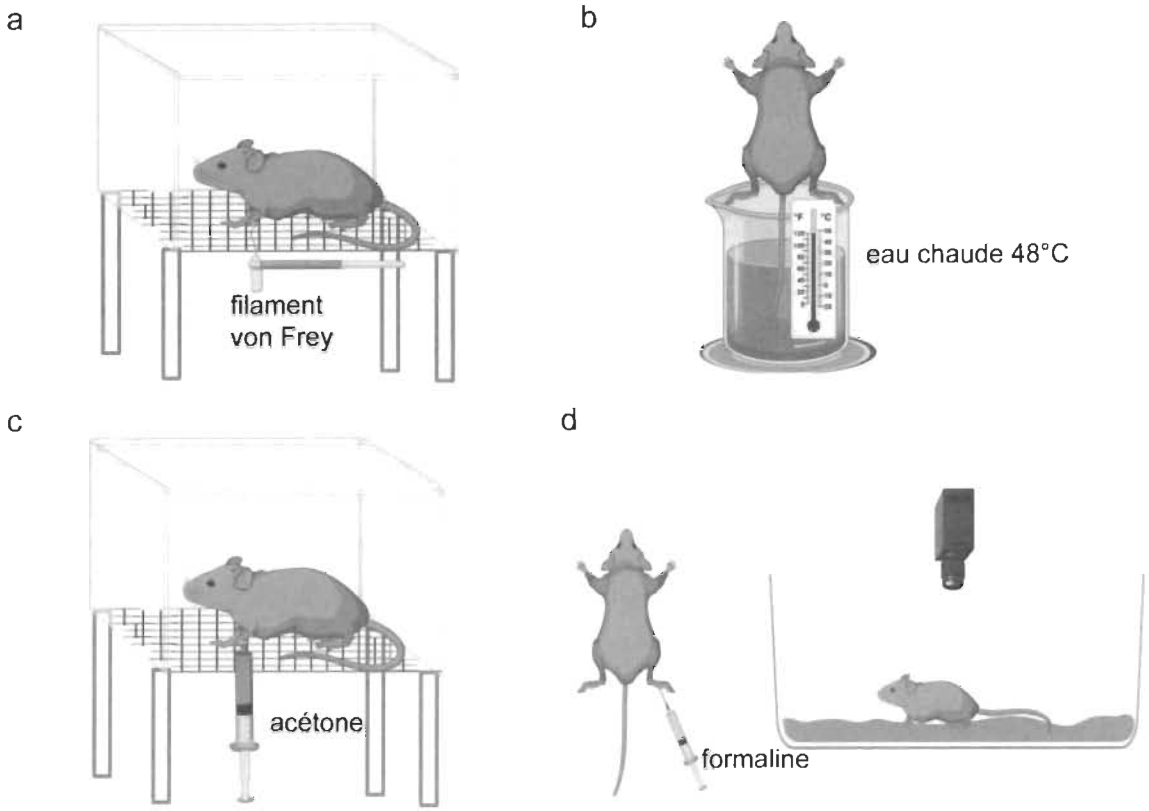


Figure 1-8 Tests de douleur

a: test de von Frey, b: test de water tail-flick, c: test d'acétone, d: test de formaline

I.3.3 L'IRM structurelle

Nous avons voulu déterminer les changements neuroanatomiques accompagnant la cécité. Pour ce faire, nous avons utilisé des techniques d'IRM *ex vivo* ainsi que des techniques d'histologie et d'immunohistochimie.

Nous avons examiné les différences cérébrales entre les souris aveugles et les souris voyantes par la technique d'IRM structurelle *ex vivo* à haute résolution (Bruker 7T 70/30 USR). L'IRM nous permet d'examiner les différences anatomiques cérébrales entre les souris voyantes et les souris aveugles dans l'ensemble du cerveau. L'avantage de la technique IRM est la possibilité de scanner un grand nombre de souris et d'obtenir les mesures de toutes les structures cérébrales rapidement (voir les détails des analyses IRM dans le chapitre IV).

I.3.4 L'histologie

I.3.4.1 Mesures histologiques

Certaines régions d'intérêt (l'amygdale, les cortex visuels, le cortex auditif et les collicules supérieurs) ont été mesurées par des techniques histologiques. Les mesures histologiques sont plus précises que les mesures IRM et permettent d'analyser plus en détails les différences volumétriques entre les groupes de souris. L'analyse histologique des cortex visuels et des collicules supérieurs, par exemple, nous permet non seulement d'obtenir des mesures volumétriques précises, mais aussi d'examiner les détails des couches cellulaires afin d'identifier les couches altérées qui causeraient l'altération du volume total de la structure. Les mesures anatomiques par histologie sont donc complémentaires aux analyses IRM et permettent d'examiner de plus près les changements neuronaux dans les structures. Cependant, contrairement à l'IRM, l'histologie n'est pas la technique de choix pour l'analyse de l'ensemble du cerveau.

I.3.4.2 La protéine c-Fos

L'IRM fonctionnelle (IRMf) est d'une certaine complexité chez la souris. À cause de sa petite taille, il est difficile d'obtenir des résultats d'imagerie fonctionnelle robustes et spécifiques. En outre, l'IRMf présente des coûts non négligeables. Donc, afin d'examiner l'activation cérébrale fonctionnelle chez la souris, on peut avoir recours à des techniques d'immunohistologie. Dans la deuxième étude de cette thèse, nous avons analysé l'activation cérébrale fonctionnelle dans l'amygdale et le CeA induite par la douleur suite à l'injection de la formaline en analysant le marquage de la protéine c-Fos.

Le c-Fos est un facteur de transcription synthétisé suite à un signal activateur. Suite à une stimulation, le proto-oncogène c-Fos est activé, transcrit en ARNm et traduit en protéine c-Fos. Avec le facteur c-jun, la protéine c-Fos forme le complexe transcriptionnel AP-1 (Herrera and Robertson, 1996). L'expression de la protéine c-Fos est rapide, robuste et réversible. Comme son activité dans les tissus non-stimulés est extrêmement faible, sa quantification suite à un signal activateur reflète l'activité cellulaire induite par la stimulation dans le tissu testé. La protéine c-Fos a été largement utilisée comme marqueur fonctionnel de l'activité neuronale

(Bullitt, 1990; Herrera and Robertson, 1996; Harris, 1998; Martinez, et al., 2002). Sa révélation est possible en utilisant un protocole d'immunohistochimie à double marquage adapté.

Chapitre II Article 1 - Better olfactory performance and larger olfactory bulbs in a mouse model of congenital blindness

Touj. S., Bronchti. G., Piché. M., Al Ain. S (To be submitted)

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Abstract

It is well established that early blindness results in enhancement of the remaining non-visual sensory modalities accompanied by functional and anatomical brain plasticity. While auditory and tactile functions have been largely investigated, the results regarding olfactory functions remained less explored and less consistent. In the present study, we investigated olfactory function in blind mice using three tests: the buried food test, the olfactory threshold test and the olfactory performance test. The results indicated better performance of blind mice in the buried food test and odor performance test while there was no difference in the olfactory threshold test. Using histological measurements, we also investigated if there was anatomical plasticity in the olfactory bulbs, the most salient site for olfactory processing. The results indicated a larger volume of the olfactory bulb driven by larger glomerular and granular layers in blind mice compared to sighted mice. Structural plasticity in the olfactory bulbs may underlie the enhanced olfactory performance in blind mice.

Introduction

Early sensory deprivation, such as in the case of early blindness, results in several behavioral adaptations. Among these, enhancement of the remaining sensory modalities is observed. Early blind people rely more on their remaining senses to assess their environment and avoid danger, and studies showed that they perform better than sighted controls in non-visual tasks. In fact, previous studies showed that blind individuals have better tactile acuity (Grant, et al., 2000; Van Boven, et al., 2000), better linguistic performance (Röder, et al., 2002; Amedi, et al., 2003) as well as better auditory spatial acuity, sound threshold and sound discrimination (Muchnik, et al., 1991; Voss, et al., 2004; Voss, 2016). Few studies focused on behavioral impacts of early blindness on olfactory functions, and the results of these studies were not always consistent. While many studies showed no advantage of blind individuals in olfactory threshold and discrimination abilities (Smith, et al., 1993a; Schwenn, et al., 2002; Sorokowska and Karwowski, 2017), other studies showed superior olfactory skills in blind subjects (Cuevas, et al., 2009; Beaulieu-Lefebvre, et al., 2011; Comoglu, et al., 2015). Using the Sniffn' Sticks test, Çomoğlu et al. showed that blind subjects have better odor discrimination and lower odor threshold than sighted controls (Comoglu, et al., 2015). A study by Cuevas et al. (2009) evidenced better performance in odor identification and odor discrimination in blind participants (Cuevas, et al., 2009). Another study showed that early blind children performed better and faster in free odor identification (Rosenbluth, et al., 2000). Moreover, Beaulieu-Lefebvre et al. (2011) showed that early blind participants had lower odor detection thresholds compared to sighted controls while there was no difference between the two groups in odor discrimination and odor identification tasks (Beaulieu-Lefebvre, et al., 2011). However, the number of the studies conducted in humans is still not sufficient to draw a clear conclusion. . Besides, studies showed that several factors related to daily life, like physical activity, might influence perceptual properties in the blind (Seemungal, et al., 2007). This underlies the importance to test olfactory performance in animal models where environmental factors and daily activities are controlled and are similar between blind and sighted animals. Only one study tested olfactory function in in rodents. In this study, Zhou et al. showed enhanced olfactory perception in rodents after one week of visual deprivation (Zhou, et al., 2017). This enhanced olfactory performance was accompanied by increased local field potentials in the olfactory bulbs

(OB) and the piriform cortex (Zhou, et al., 2017). The OB are the first site for processing olfactory information and play a key role in olfactory function (Mori, et al., 1999). Moreover, studies evidenced that the OB are very plastic (Huart, et al., 2013) and that their size varies based on olfactory sensitivity and training and is decreased following olfactory disorders (Yousem, et al., 1999; Mueller, et al., 2005; Rombaux, et al., 2006a; Rombaux, et al., 2006b). Very few studies focused on anatomical changes in the olfactory system following early blindness. One study conducted in early blind individuals showed an increase of the OB volume that correlated with enhanced olfactory functions such as odor discrimination and odor-free identification scores (Rombaux, et al., 2010).

The aim of the present study was to assess olfactory performance and examine anatomical changes in the OB in a mouse model of congenital blindness. The ZRDBA mouse strain allows investigation of the impacts of congenital blindness on behavioral, functional and anatomical levels. Three tests were used to assess olfactory performance in blind and sighted mice: the buried food test, the olfactory threshold test and the olfactory performance test. Structural changes of the OB and its layers were examined using histological analysis.

Based on previous findings in humans, we hypothesized that blind mice would have better olfactory performance compared to sighted mice. We also hypothesized that the OB volume would be larger in blind mice compared to sighted mice.

Methods

Animals

A total of 133 adult mice (10-12 weeks old) were used in this study, including 66 sighted and 67 blind ZRDBA mice, with each group comprising males and females. Different animal groups were used for the different tests. For the buried food test, 30 sighted mice and 33 blind mice were used. For the odor threshold test, 15 sighted mice and 14 blind mice were used. For the odor performance test, 11 sighted mice and 10 blind mice were used. Ten sighted mice and 10 blind mice were used for histological measurement. Animals were kept in local facilities with a light/dark cycle of 14 h/10 h. Animals were provided with food and water ad libitum. All experimental procedures were approved by the animal care committee of " Université du

Québec à Trois-Rivières", in accordance with the guidelines of the Canadian Council on Animal Care.

ZRDBA mouse strain

The anophthalmic ZRDCT mouse strain was first described by Chase (Chase and Chase, 1941; Chase, 1942). A mutation on chromosome 18 in the Rx/Rax gene results in the absence of eyes and optic nerves as well as the retinal afferent to the hypothalamus (Tucker, et al., 2001). The ZRDBA mouse strain was created by crossbreeding the ZRDCT anophthalmic strain with the sighted DBA-6 strain. In this strain, half of the littermates homozygous for the Rx/Rax gene are born blind and the other heterozygous half are born normally sighted. Sighted and blind mice are housed in mixed cages in a local facility.

Olfactory performance assessment

Buried food test

The design of this test was based on previous reports (Yang and Crawley, 2009b; Canugovi, et al., 2015). The test was conducted in a dark room with only a red light source. We used the food chow pellets that mice were used to eating (Charles River, Sherbrooke, Canada), which removed the need for pretraining for an exogenous food odor. Animals were habituated to the testing cage for 30 min/day for 5 days. Mice were deprived of food 16 hours before the test and only had access to water. They were allowed 15 min of acclimation in a clean cage with 3-4 cm depth clean bedding. The animal was taken out of the cage and the food chow was then buried at a 1-cm depth in a random corner. The mouse was placed again in the testing cage and its behavior was recorded by video (see Fig.1-a). The latency and distance travelled in the cage before finding the food pellet were calculated using Ethovision XT software (Noldus, Virginia, USA) and compared between the two groups.

Olfactory threshold test

The protocol was adapted from Witt et al. 2009 (Witt, et al., 2009). The test was conducted in a dark room with only a red light source. The olfactory threshold test allows testing of mouse olfactory sensitivity by determining the lowest concentration that animals can detect. Mice were habituated to the testing cage 30 min/day for five days prior to the experiment. Each mouse was tested for each dilution separately in a cage without bedding. Mice were considered in the odor exploratory area if their heads were within 1 cm of the filter paper. Serial dilutions (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}) of cinnamon scent (Cinnamaldehyde, Sigma-Aldrich, natural, ≥95%, FG; Molecular Weight 132.16) and rose scent (Phenethyl alcohol, Sigma-Aldrich, natural, ≥95%, FG; Molecular Weight 122.16) in water were presented sequentially to each mouse (see Fig.1-b). Each scent was presented, and the mouse behavior was recorded for 3 min with 1 min intervals between trials. In the first two trials, animals were presented with only water. Exploratory time near the scent-containing zone was recorded and calculated for each dilution from the videos using Ethovision XT software (Noldus, Virginia, USA).

Olfactory performance test

This protocol was adapted from Witt et al. 2009 (Witt, et al., 2009) and Canugovi et al. 2015 (Canugovi, et al., 2015). The test was conducted in a dark room with only a red light source. This test examines the mouse's ability to differentiate various smells as an essential survival instinct. Good olfactory performance allows animals to recognize consumable food from toxic material (Yang and Crawley, 2009a). Filter paper impregnated with 50 µl of each odor was presented to each animal simultaneously with filter paper impregnated with water with a 3 cm spacing (see Fig.1-c). Mice were considered in the odor/water exploratory area if they were within 1 cm of the filter paper. The odor is considered attractive if its exploratory time is higher than that of water, neutral if its exploratory time is equal to that of water and aversive if its exploratory time is less than that of water. Three odors were tested based on the protocol by Canugovi et al. (Canugovi, et al., 2015): 10% w/v vanillin (Sigma-Aldrich, St. Louis, MO), 10% w/v peanut butter and 10% v/v 2-methylbutyricacid (2MB) (Sigma-Aldrich). Mice were tested for 3 min with each scent with a 1 min interval. Exploratory times for water and the odor were recorded and calculated from videos using Ethovision XT software (Noldus, Virginia, USA).

Water exploratory time was subtracted from the total exploratory time for each scent, and the obtained values were compared between mouse groups.

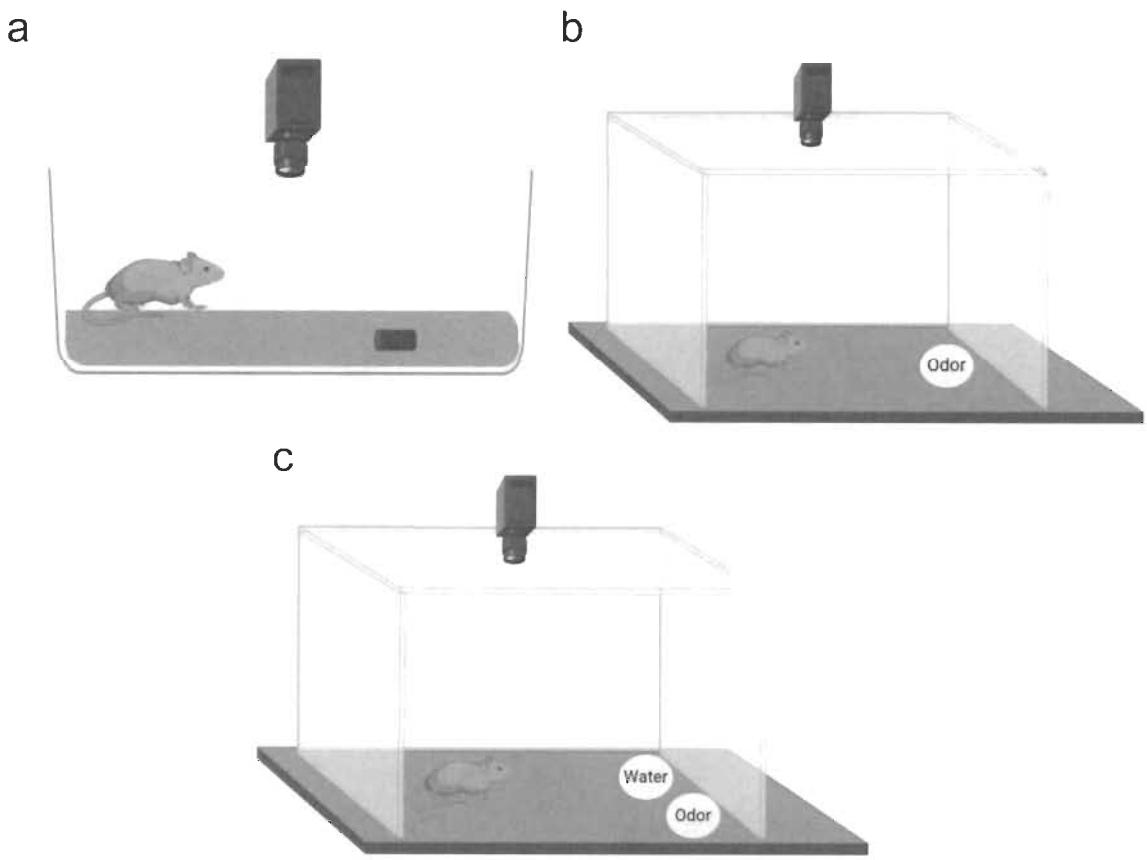

Figure 2-1 Olfactory performance assessment tests

a- Buried food test; b- Olfactory threshold test; c- odor performance test.

Histological measurements

Mice were deeply anesthetized with 4% isoflurane in 100% oxygen with a delivery rate of 4 l/min through the heart with 30 ml of PBS solution (Phosphate Buffered Saline 0.1 M) followed by 30 ml of 4% paraformaldehyde solution (Paraformaldehyde, powder, 95%, Sigma Aldrich, Missouri, USA) at a flow rate of 1.0 ml/min. Immediately after perfusion, the head was removed and placed in a 4% paraformaldehyde solution at 4 °C overnight. Brains were collected and post-fixed in formalin for 24 hours and cryoprotected in a 30% sucrose solution. Coronal sections of 50 µm thickness were collected using a freezing microtome (Leica VT1000S, Leica microsystems, Wetzlar, Germany), mounted on gelatin-coated slides, dried, and processed for Nissl staining, dehydrated, and cover-slipped. The volumes of the right and left OB were measured on Nissl-stained brain sections under an Olympus BX50W1 microscope coupled to a CCD camera (Optronix, MicroBrightField, Williston, VT, U.S.A.). Delineation of the OB was performed with Neurolucida Software (MicroBrightField, Williston, VT, U.S.A.). The experimenter was blind to the mouse phenotype. Delineations were based on stereotaxic coordinates by Paxinos and Watson (1998). Sections were collected for each brain between Bregma +5.56 mm and Bregma +3.56 mm. The contours of each olfactory bulb and its different layers, namely the glomerular (gl), outer plexiform (opl), mitral (mi), inner plexiform (ipl) and granular layers (gr), were delineated on every other section (every 100 µm) for a total of 20 sections. The external limit of the glomerular layer was considered as the external limit of the OB. To calculate the volumes of OB and the different layers, the surface of these 20 sections was multiplied by 100 µm to account for the inter-section gap. These volumes were then summed to obtain the total volume of each olfactory bulb.

Statistical analyses

Statistical analyses were performed with Statistica v13.0 (Dell Inc., Tulsa, OK, USA). We verified that all data were normally distributed using Kolmogorov-Smirnov & Lilliefors tests. Latencies and distance in the buried food test were compared between groups using a Student's t-test. Odor threshold and odor performance were compared between groups using mixed ANOVA (see details for each analysis in the results section). OB volumes were compared

between groups using two way ANOVA with group and side as between-subject factors. Volumes of OB layers were compared between groups using a mixed ANOVA with group, brain side and layer as between subject factors. Values of $p \leq 0.05$ were considered statistically significant. Effect sizes are reported based on partial eta squared ($\eta 2p$).

Results

Buried food test

Average latencies to find food pellets were approximately three times shorter in blind compared to sighted mice (34.1 vs 61 sec; t=2.73, p=0.007; Fig. 2). To ensure that the results were not due to differences in exploratory behavior, travel distances were compared and no differences were observed (blind mice=243.6 cm, sighted mice=286 cm; t=0.62, p=0.53).

Figure 2-2 Buried food test

The latency before finding a buried food pellet was shorter in blind mice compared to sighted mice (Blind mice=34.1 s, sighted mice=61 s) (p=0.007)

Olfactory threshold test

Results were compared between groups using mixed ANOVAs with group and odor concentration as a between subject factors.

For rose scent, sniffing durations were not significantly different between groups across odor concentrations (interaction: $F_{8,20}$= 0.5; p=0.85; η^2_p =0.01). There was no significant effect of group ($F_{1,26}$= 0.0003; p=0.98; η^2_p =0.0001). There was a significant effect of odor concentration ($F_{8,20}$= 13.97; p<0.001; η2p =0.34). LSD Fisher post hoc test revealed a significant difference between odor concentration 10^{-7} and all other odor concentrations (all p <0.001). This indicates that the odor was detected by both groups at the dilution 10^{-7} followed by a decrease in exploratory time indicating, most likely, a habituation effect for odor concentrations 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} (see Fig. 3-a).

For cinnamon scent, sniffing durations were not significantly different between groups across odor concentrations ($F_{8,20}$= 0.45; p=0.45; η^2_p =0.01). There was no significant effect of group ($F_{1,26}$= 1.32; p=0.25; η^2_p =0.03). There was a significant effect of odor concentration ($F_{8,20}$= 14.78; p<0.001; η^2p =0.30). LSD Fisher Post hoc test revealed a significant difference between odor concentration 10^{-5} and all other odor concentrations (all p <0.001). This indicates that the odor was detected by both groups at the dilution 10^{-5}, followed by a decrease in exploratory time indicating, most likely, a habituation effect for odor concentrations 10^{-4} and 10^{-3} (see Fig. 3-b).

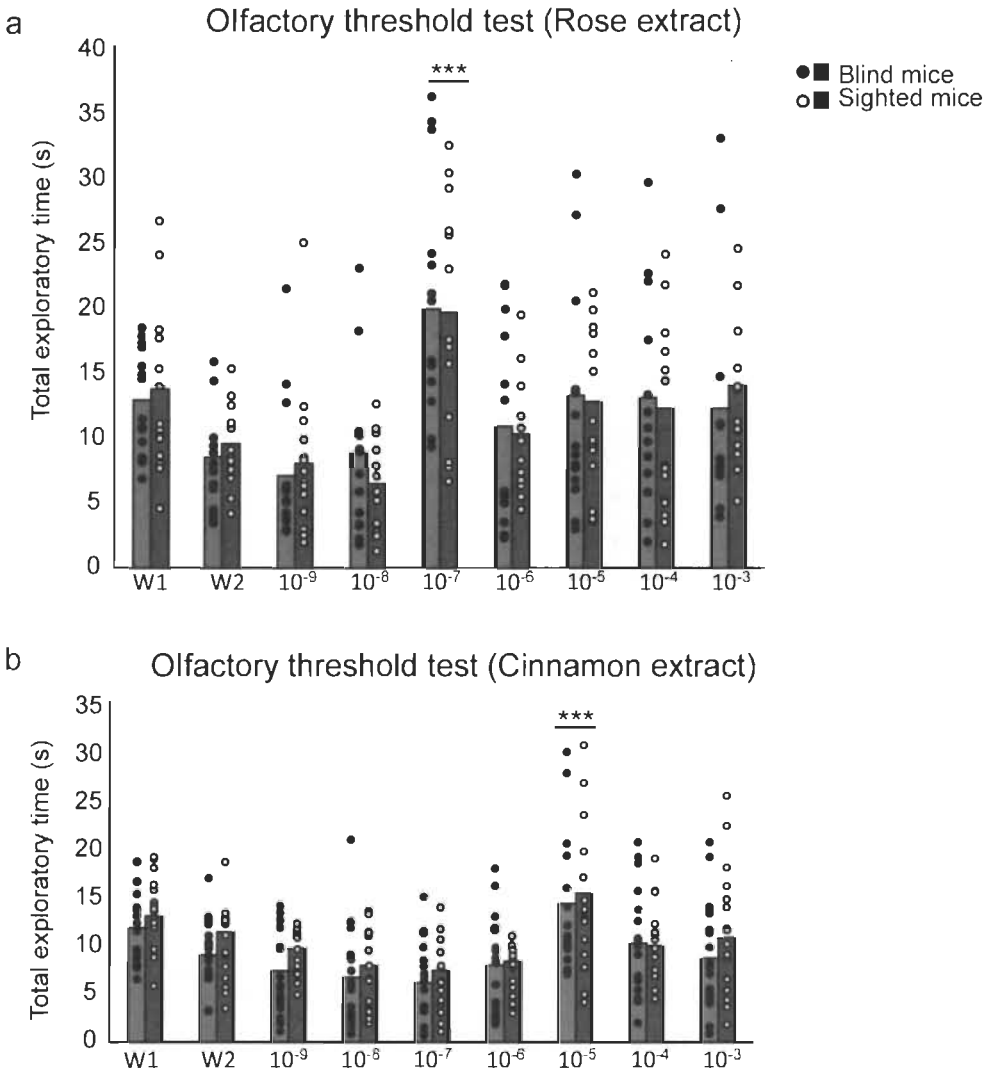

Figure 2-3 Olfactory threshold test

a Olfactory threshold test using rose scent. In the two first trials, animals were exposed to water (W1 and W2). The next 7 trials were conducted using serial dilutions of rose scent (10^{-9} to 10^{-3}). There was no significant difference between groups for all concentrations of rose scent. There was a significant difference between the 10^{-7} concentrations and all other concentrations (all $p<0.001$), indicating that the odor was detected at 10^{-7} in both mouse groups. b Olfactory threshold test using cinnamon scent. In the two first trials, animals were exposed to water (W1 and W2). The next 7 trials were conducted using serial dilutions of cinnamon scent (10^{-9} to 10^{-3}). There was no significant difference between groups for all concentrations of cinnamon scent.

*There was a significant difference between the 10^{-5} concentration and all other concentrations (all $p<0.001$), indicating that the odor was detected at 10^{-5} in both mouse groups *** $p<0.001$.*

Odor performance test

All animals were exposed to three odors, vanilla scent, peanut butter scent and 2MB, in a random order.

Mixed ANOVA revealed a significant effect of group ($F_{1,19} = 11.991$; $p = 0.003$; $\eta^2_p = 0.38$), odor ($F_{2,38} = 114.15$; $p < 0.001$; $\eta^2_p = 0.85$), and the interaction odor*group ($F_{2,38} = 54.14$; $p < 0.001$; $\eta^2_p = 0.74$). LSD Fisher post hoc test was conducted to assess group differences for the three odors used in the test. The results showed a significant difference in the total exploratory time (with water subtracted) between the sighted mice and blind mice for vanilla scent ($p= 0.001$), peanut butter ($p < 0.001$) and 2MB ($p = 0.001$), indicating better performance of blind mice with all three odors (see Fig. 4).

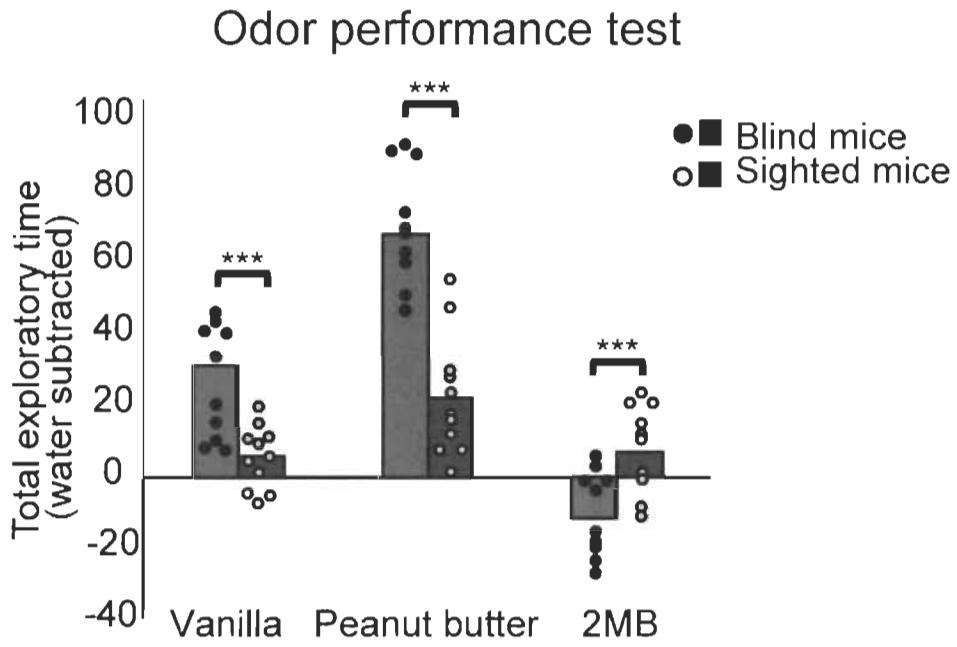

Figure 2-4 Odor performance test

*The performance of blind mice was significantly better than that of sighted mice in tests using vanilla scent ($p<0.001$), peanut butter scent ($p<0.001$) and 2MB scent ($p=0.001$) ***$p<0.001$.*

Histological measurements

A representative section of the anatomical delineation of the OB and of its respective layers is shown in Fig. 5. The OB volumes were compared between groups and hemispheres with a two-way ANOVA with group and brain side as between-subject factors (see Fig. 6). The OB volume was larger in blind mice compared with sighted mice (main effect: $F_{1,18}=11.16$, $p=0.003$; $\eta^2_p=0.38$). This effect was not significantly different between sides (interaction: $F_{1,18}=0.02$, $p=0.61$; $\eta^2_p=0.01$) (see Fig. 6).

The OB layers volumes were compared between groups and brain sides with a mixed ANOVA with group, brain side and layer as between subject factors (see Fig. 7).

The volume was different between groups across layers (interaction: $F_{1,72}=5.53$, $p<0.001$; $\eta^2_p=22.14$). This effect was not significantly different between brain sides (interaction:

$F_{1,18}$=0.26, p=0.6; η^2_p=0.26). LSD Fisher post hoc test revealed a significant difference between groups for the glomerular layer volume (p<0.001) (see Fig 7-a) and the granular layer volume (p=0.003) (see Fig 7-e). There was no significant difference between groups for the outer plexiform layer (p=0.25) (see Fig 7-b), the mitral layer (p=0.54) (see Fig 7-c) and the inner plexiform layer (p=0.74) (see Fig 7-d).

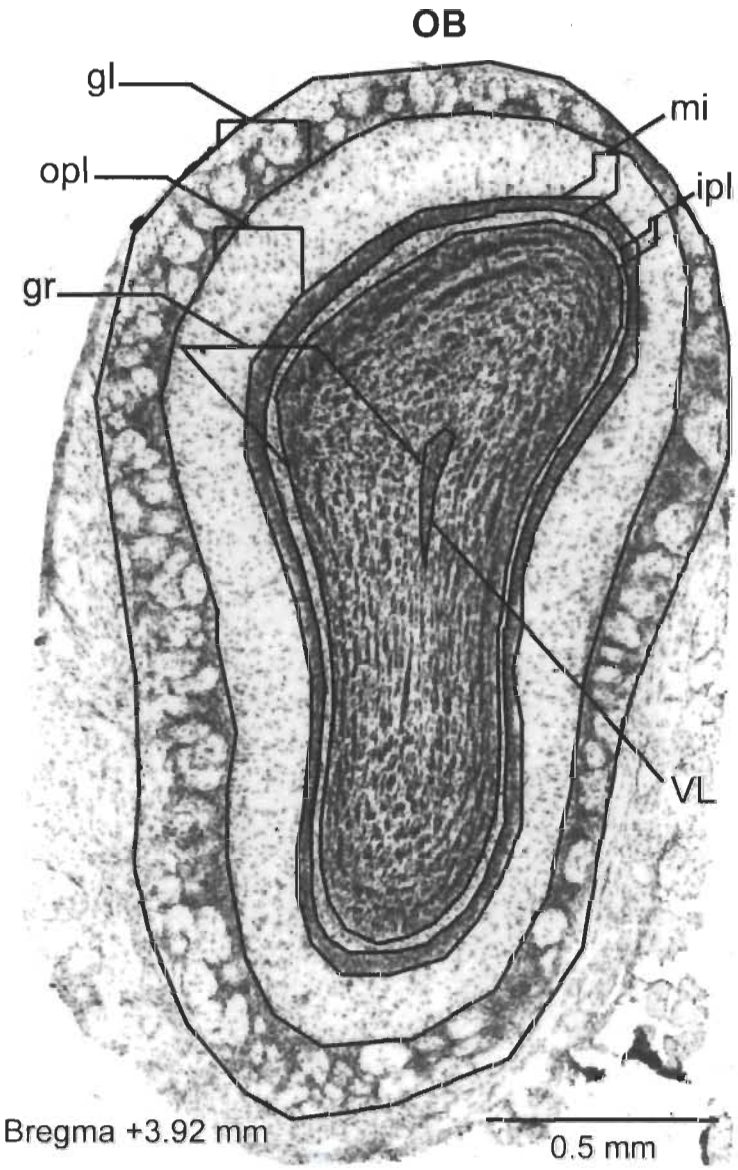

Figure 2-5 Photomicrograph showing delineation of the left OB

Photomicrographs of the delineation of the OB at Bregma +3.92 mm based on stereotaxic coordinates by Paxinos and Franklin (1998) (Paxinos and Franklin, 1998). ***OB*** *Olfactory bulbs*

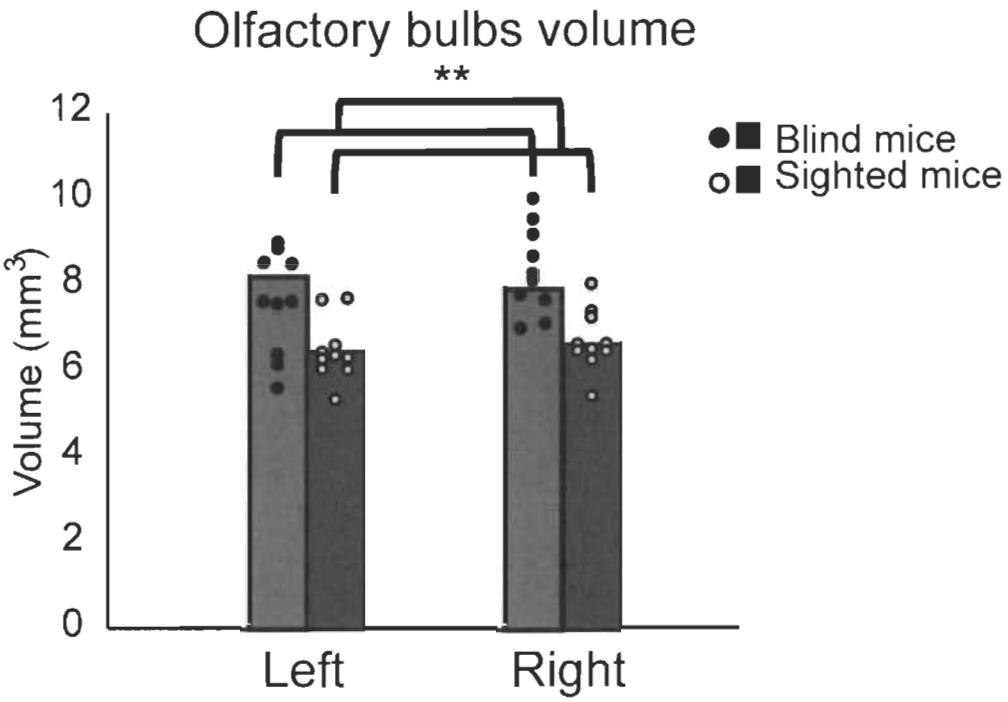

Figure 2-6 Larger olfactory bulb volume in blind mice

The volumes of the left and right OB were significantly larger in blind mice compared with those of sighted mice ($p=0.002$) $**p<0.01$.

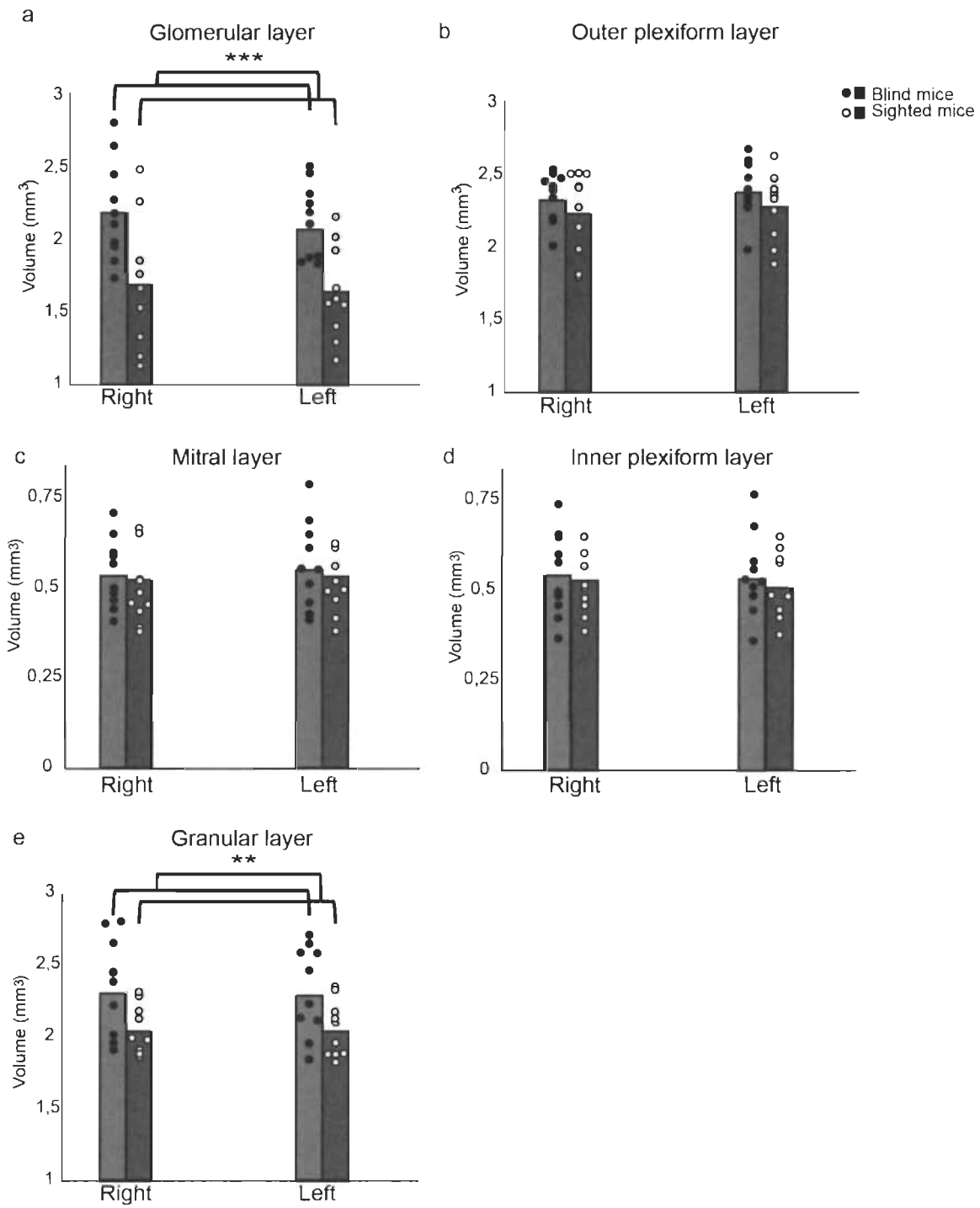

Figure 2-7 Larger glomerular and granular layers volume in blind mice

a The volumes of the left and right glomerular layer were larger in blind mice compared with those of sighted mice ($p < 0.001$) b No volume difference was observed for the outer plexiform layer between groups ($p=0.25$) c No volume difference was observed for the mitral layer

*between groups (p=0.54) d No volume difference was observed for the inner plexiform layer between groups (p=0.74) e The volumes of the left and right granular layer were larger in blind mice compared with those of sighted mice (p=0.003) **p<0.01 **p<0.001.*

Discussion

This is the first study to examine olfactory performance in a congenitally blind animals. In the present study, we used a mouse model of congenital blindness. Our findings indicate enhanced olfactory performance in blind mice compared with sighted mice. Blind mice also showed larger OB volume compared with sighted mice. Blind mice also showed larger OB volumes driven by larger glomerular and granular layers compared with sighted mice.

In the present study, we used three different behavioral tests to assess olfactory performance in blind mice: the buried food test, the olfactory threshold test and the odor performance test. Blind mice had lower latencies in the food seeking test and were better at the odor performance test, as they had lower exploratory time for the aversive odor and higher exploratory time for the neutral and attractive odors than sighted mice. There was no difference between groups in the odor threshold test.

While several studies have not shown any advantage of blind over sighted participants in olfactory performance (Smith, et al., 1993b; Luers, et al., 2014; Manescu, et al., 2018; Sorokowska, et al., 2018; Sorokowska, et al., 2019), others have evidenced enhanced olfactory function in blind subjects (Cuevas, et al., 2009; Rombaux, et al., 2010; Beaulieu-Lefebvre, et al., 2011; Renier, et al., 2013; Comoglu, et al., 2015; Gagnon, et al., 2015). The discrepancy of the results between studies is probably due to differences in the testing procedures. Studies conducted in humans used different tests ranging from home-made dilutions and odorants to validated tests like the sniffin'Sticks, as proposed by Lefebvre et al. (Beaulieu-Lefebvre, et al., 2011). The sample size also varies greatly between studies, and the age of blindness onset as well as the age of the participants are not controlled in most studies. This emphasizes the importance to test olfactory performance in animal models of early blindness where these factors can be controlled.

Blind people and animals pay more attention to non-visual cues in their daily life including the olfactory ones (Cuevas, et al., 2009; Gagnon, et al., 2015). This leads to an enhanced processing of olfactory information in their environment. In the present study, blind mice had higher exploratory durations with positive valence odors (peanut butter and vanilla scents) and lower exploratory durations with the negative valence odor of 2MB compared to sighted mice. Higher exploratory time with the first two odors could reflect higher attentional processes where blind mice tend to further explore and analyze newly introduced positive valence odors in their environment. Their lower 2MB exploratory time could indicate their superior odor discrimination ability. It could also indicate their enhanced ability to avoid danger in their environment as a compensatory survival skill. Taken together, our results of the food seeking test and the olfactory performance test might indicate superior odor awareness and odor discrimination in blind mice compared to sighted mice.

Our results are in line with studies conducted in humans showing that individuals with early blindness have higher odor awareness (Beaulieu-Lefebvre, et al., 2011) and better odor discrimination and identification (Murphy and Cain, 1986; Rosenbluth, et al., 2000; Rombaux, et al., 2010; Gagnon, et al., 2015). Our results are also consistent with a study from Zhou et al. showing that postnatal visual deprivation in mice results in better performance in an odor performance test and lowers the latency of food seeking in the buried food test (Zhou, et al., 2017). In accordance with our results, most studies conducted in humans showed no advantage of blind subjects in odor threshold (Rosenbluth, et al., 2000; Wakefield, et al., 2004; Luers, et al., 2014; Cornell Karnekull, et al., 2016; Sorokowska, 2016). Only three studies evidenced a lower olfactory threshold in blind individuals (Cuevas, et al., 2010; Beaulieu-Lefebvre, et al., 2011; Comoglu, et al., 2015). This is also in line with behavioral results examining other sensory modalities, such as auditory function, in blind humans. Studies of auditory performance showed no advantage of blind subjects in auditory absolute threshold tasks (Collignon, et al., 2006; Collignon and De Volder, 2009; Nilsson and Schenkman, 2016). In contrast, blind individuals show better sound discrimination and categorization (Muchnik, et al., 1991; Gougoux, et al., 2004; Hötting and Röder, 2009; Wan, et al., 2010).

Our results suggest that blindness does not affect olfactory threshold, but it enhances odor discrimination and odor awareness as an adaptive mechanism important for survival.

Another explanation for the absence of difference in olfactory threshold between blind and sighted subjects is the difference in olfactory processing between the olfactory threshold and other olfactory components. Contrary to odor discrimination and odor identification that require high order olfactory processing, the measure of olfactory threshold does not involve complex cognitive function (Hedner, et al., 2010; Sorokowska, et al., 2013). The olfactory threshold task seems to depend on peripheral olfactory function (Whitcroft, et al., 2017; Sorokowska, et al., 2018).

Enhancement of other non-visual sensory modalities was evidenced in blind subjects. Studies showed that blind individuals have higher tactile acuity than sighted controls (Van Boven, et al., 2000; Goldreich and Kanics, 2003; Chebat, et al., 2007; Legge, et al., 2008; Wong, et al., 2011). These behavioral enhancements are usually closely related to functional and structural cerebral plasticity in blind individuals. For instance, it was shown that early blind participants have enhanced spatial navigation skills associated with a larger hippocampal volume (Fortin, et al., 2008). In blind mice, it was shown that pain hypersensitivity is accompanied by enlargement of the amygdaloid complex volume, a structure involved in pain perception and modulation (Touj, et al., 2019). Zhou et al. showed that in visually deprived mice, enhanced olfactory performance was accompanied by increased functional activity in the OB and piriform cortex (Zhou, et al., 2017). Moreover, Rombaux et al. showed that enhanced olfactory performance in individuals with early blindness is associated with a larger olfactory bulb volume (Rombaux, et al., 2010).

In accordance with these data, our histological analysis indicated a larger OB volume in blind mice compared to sighted mice. Studies evidenced that the OB are very plastic and can undergo neural and structural changes as a result of environmental changes (Rosselli-Austin and Williams, 1990; Huart, et al., 2013; Araneda, et al., 2016; Zhou, et al., 2017). Studies conducted in humans evidenced that the OB volume correlates with olfactory function (Mueller, et al., 2005; Huart, et al., 2013; Hummel, et al., 2013; Mazal, et al., 2016). In a study conducted in healthy subjects, Buschhüter et al. (2008) showed that the volume of the OB correlates with olfactory performance (Buschhuter, et al., 2008) and that the OB volume is decreased with age, leading to decreased overall olfactory performance (Buschhuter, et al., 2008). In a recent review, Mazal et al. (2016) showed that the OB volume did not correlate specifically with an olfactory

component (threshold, identification, or discrimination) but correlated with overall olfactory performance (Mazal, et al., 2016). Furthermore, a volume decrease of the OB correlating with reduced olfactory performance was evidenced in several clinical conditions such as trauma, infections and neurodegenerative diseases (Mueller, et al., 2005; Rombaux, et al., 2006a; Rombaux, et al., 2006b; Jiang, et al., 2009).

A detailed analysis of the different OB layers evidenced larger volumes of the glomerular and granular cell layers in blind mice. The enlargement of these layers underlies the larger OB volume in blind mice. Glomerular and granular cell layers play an important role in olfactory function. A recent study showed that these cells express the oncofetal trophoblast glycoprotein 5T4 gene required for odor detection and discrimination behaviors (Takahashi, et al., 2018). Another study evidenced that broad activation of the glomerular layer cells enhances subsequent olfactory responses (Woo, et al., 2007). Moreover, disinhibition of granule cells was shown to accelerate odor discrimination in mice (Nunes and Kuner, 2015).

In the present study, the enlargement of these olfactory structures in the blind mouse brain may be a compensatory mechanism following early visual loss and could explain the enhanced olfactory performance observed in blind mice. However, the enhanced olfactory abilities and associated brain plasticity might be experience-dependent and caused by training rather than a direct consequence of blindness itself. Evidence of the effect of training on brain plasticity was shown previously (Zatorre, et al., 2012). In fact, blind people rely more on their remaining senses to survive and avoid danger. For instance, blind individuals score higher on odor awareness scales in most studies (Beaulieu-Lefebvre, et al., 2011). This overuse might consequently lead to structural brain plasticity and thus behavioral enhancement.

Future studies will allow investigation of the underlying mechanisms of OB plasticity and the establishment of a causal relationship between brain plasticity and the behavioral adaptations observed in the present study. We also do not exclude that other brain regions involved in olfactory function that were not examined may present structural alterations and contribute to behavioral changes in blind mice. This should be investigated in subsequent studies.

Conclusion

In conclusion, the present study provides evidence that olfactory performance is enhanced in early blind mice. Histological measurements showed larger OB volume in early blind mice driven by larger volumes of the glomerular and granular cell layers. The present results suggest that the plasticity of the OB could underlie the enhanced olfactory performance evidenced in blind mice.

Abbreviations

2MB 2-Methylbutyricacid; **gl** glomerular layer; **gr** granular layer; **ipl** inner plexiform layer; **mi** mitral layer; **OB** Olfactory Bulbs; **opl** outer plexiform layer; **PBS** Phosphate Buffered Saline; **VL** Lateral Ventricle.

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Chapitre III Article 2 - Pain Hypersensitivity is Associated with Increased Amygdala Volume and c-Fos Immunoreactivity in Anophthalmic Mice

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Abstract

It is well established that early blindness results in brain plasticity and behavioural changes in both humans and animals. However, only a few studies have examined the effects of blindness on pain perception. In these studies, pain hypersensitivity was reported in early, but not late, blind humans. The underlying mechanisms remain unclear, but considering its key role in pain perception and modulation, the amygdala may contribute to this pain hypersensitivity. The first aim of this study was to develop an animal model of early blindness to examine the effects of blindness on pain perception. A mouse cross was therefore developed (ZRDBA mice), in which half of the animals are born sighted and half are born anophthalmic, allowing comparisons between blind and sighted mice with the same genetic background. The second aim of the present study was to examine mechanical and thermal pain thresholds as well as pain behaviors and pain-related c-Fos immunoreactivity induced by the formalin test in the amygdalas of blind and sighted mice. Group differences in amygdala volume were also assessed histologically. Blind mice exhibited lower mechanical and thermal pain thresholds and more pain behaviors during the acute phase of the formalin test, compared with sighted mice. Moreover, pain hypersensitivity during the formalin test was associated with increased c-Fos immunoreactivity in the amygdala. Furthermore, amygdala volume was larger bilaterally in blind compared with sighted mice. These results indicate that congenitally blind mice show pain hypersensitivity like early blind individuals and suggest that this is due in part to plasticity in the amygdala.

Introduction

Vision is crucial for interacting with the environment, including for behavioral adaptation to protect body's integrity. Thus, the impact of blindness on brain plasticity, sensory functions, and behavior were examined in human and animal studies. Early blindness causes plasticity in the brain including rewiring of cortico-cortical connections and volumetric changes in cortical and subcortical regions (Bronchti, et al., 2002; Fortin, et al., 2008; Charbonneau, et al., 2012; Collignon, et al., 2013; Kupers and Ptito, 2014). In humans, these changes are closely related to behavioral changes (Theoret, et al., 2004) including enhanced linguistic performance (Amedi, et al., 2003), improved auditory (Voss, 2016) and tactile (Goldreich and Kanics, 2003) discrimination, as well as supranormal spatial navigation (Fortin, et al., 2008).

Although sensory systems have been well investigated in the blind, only a few studies have examined the nociceptive system. For example, male and female adult participants with congenital blindness, but not late-onset blindness, show lower heat and cold pain thresholds as well as increased pain perception of suprathreshold nociceptive stimuli compared to controls with normal vision (Slimani, et al., 2013; Slimani, et al., 2014). However, the underlying mechanisms of these differences remain to be clarified. Considering that warm and cold detection thresholds are comparable between individuals with congenital blindness and normal vision (Slimani, et al., 2013; Slimani, et al., 2014), increased thermal sensitivity in the congenitally blind may rely on higher-order or emotional brain processes.

The amygdala is a key structure for emotions, pain processing, pain modulation, and chronic pain (Fanselow and LeDoux, 1999; Fanselow and Gale, 2003; Phelps and LeDoux, 2005; Veinante, et al., 2013; Neugebauer, 2015; Kato, et al., 2018; Miyazawa, et al., 2018). Behavioral and physiological studies have shown functional changes in the amygdala in different pain conditions in humans and animal pain models (Bernard, et al., 1992; Bornhovd, et al., 2002; Berman, et al., 2006). The central nucleus of the amygdala (CeA) contains most of its responsive neurons to nociceptive stimulation. Accordingly, the CeA receives nociceptive input from the spinoparabrachial pathway (Gauriau and Bernard, 2002) and nociceptive information from the thalamus and cerebral cortex through the basolateral amygdala (BLA) (McDonald, et al., 1999; Sah, et al., 2003). In addition, animal studies have shown increased CeA activity in acute somatic pain (Bernard, et al., 1992; Neugebauer and Li, 2002),

inflammatory pain (formalin injection) (Carrasquillo and Gereau, 2007), visceral pain (Nakagawa, et al., 2003; Suwanprathes, et al., 2003; Han and Neugebauer, 2004; Sadler, et al., 2017), and neuropathic pain (Ikeda, et al., 2007; Goncalves, et al., 2008; Cooper, et al., 2018). Thus, the amygdala may play a critical role in pain hypersensitivity in congenital blindness, although this does not exclude other mechanisms.

The aim of this study was to examine pain sensitivity in anophthalmic mice and the role of the amygdala in pain sensitivity changes due to blindness. In order to avoid the confounding factor of strain differences, a mouse model was developed to obtain blind and sighted mice from the same strain. The so-called ZRDBA mouse was obtained through genetically controlled back-crossing between ZRDCT anophthalmic mice and DBA mice with normal eyes. In this cross, half of animals are born sighted (heterozygous), while the other half are born anophthalmic (homozygous). Using this model, mechanical pain thresholds (Von Frey test), thermal pain thresholds (Acetone drop and Tail-Flick tests), as well as formalin-induced pain behaviors and c-Fos immunoreactivity in the amygdala were compared between blind and sighted mice. Group differences in amygdala volume were also assessed histologically. We hypothesized that blind mice would be hypersensitive to pain compared with sighted mice. We also expected increased pain-related activity in the amygdala, especially in the CeA. In line with brain plasticity described in the blind, we also hypothesized that amygdala volume would be larger in blind compared with sighted mice.

Material and methods

Animals

A total of 70 adult ZRDBA mice (10-12 weeks old) were used for the purpose of this study (see Table 1). Forty mice were used for the formalin test, including 20 anophthalmic and 20 normally sighted mice, among which 14 mice (7 anophthalmic and 7 sighted) were used for c-Fos immunohistochemistry and histology. An additional group including 5 sighted and 5 anophthalmic mice was used as a control group for c-Fos immonureactivity (no formalin injection). A third group of 20 mice (10 anophthalmic and 10 sighted) was used to assess mechanical and thermal pain thresholds. Each group comprised males and females to ensure

generalizability of the results to both sexes (see Table 1 for details). Anophthalmic and sighted mice issued from an anophthalmic or sighted female were housed in mixed cages (anophthalmic and sighted mice) in local facilities, with a light/dark cycle of 14h/10h.

All experimental procedures were approved by the animal care committee of the Université du Québec à Trois-Rivières in accordance with the guidelines of the Canadian Council on Animal Care and the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP).

Table 3-1 Detailed sample information

	Sighted males	Sighted females	Anophtalmic males	Anophthalmic females
Group 1 (n=40)				
Formalin test	10	10	10	10
c-Fos - formalin and histological measures (among animals used for the formalin test)	4	3	3	4
Group 2 (n=10)				
c-Fos - control and histological measures	3	2	2	3
Group 3 (n=20)				
von Frey test, Acetone drop test, Hot water immersion tail flick test	5	5	5	5

ZRDBA cross

The ZRDBA mouse cross is issued from crossbreeding of the sighted DBA-6 strain and the ZRDCT anophthalmic strain. The ZRDCT mouse was first described by Chase (Chase and

Chase, 1941; Chase, 1942; Chase, 1944). These mice have orbits but lack eyes and optic nerves. In 2001 Tucker et al. (Tucker, et al., 2001) provided the genetic description of the mutant mouse ZRDCT. More than 90% of the homozygous ZRDCT mice for a major recessive determinant gene are born anophthalmic (Chase and Chase, 1941; Chase, 1942). The eyeless phenotype is due to a mutation in a recessive locus (ey1) on chromosome 18 leading to a mutation in the Rx/Rax gene. This gene is expressed in the anterior headfold, and is involved in the development of the retina, pineal gland, and hypothalamus (Tucker, et al., 2001). In addition to the lack of eyes and optic nerve, the mutation results in the absence of retinal afferents to the hypothalamus (Tucker, et al., 2001) and asymmetry of the suprachiasmatic nuclei (Laemle and Rusa, 1992) as well as a development of auditory fibers in the lateral geniculate nucleus (LGN), a thalamic relay to the visual cortex (Piche, et al., 2004; Chabot, et al., 2007). No other major brain defect is described in the ZRDCT mouse strain. Despite the lack of retinal afferents, the circadian rhythm in this mouse strain was shown to be controlled by other nonphotic stimuli (Laemle and Ottenweller, 1999).

In the ZRDBA colony developed for the purpose of the present study, about half of the littermates are born anophthalmic and the other half are born normally sighted when blind females are bred with sighted males or when sighted females are bred with blind males. The ZRDBA cross is advantageous for the study of the impacts of early blindness on pain since anophthalmic mice and littermate controls have the same genetic background.

Behavioral assessment

Von Frey test

To determine the mechanical pain threshold, von Frey filaments were used with the ascending method (Yalcin, et al., 2014). Animals were placed in transparent plastic cages with an elevated mesh floor. They were habituated to the testing cage 30 minutes prior to testing. Mechanical pain thresholds were determined for both hind paws. The test consisted of the application of von Frey filaments of increasing stiffness (0.6, 1, 1.4, 2, 4.0, and 6.0). Filaments were applied gently on the plantar surface of the hind paw until bending, then removed progressively for a total application duration of approximately 1 second. The application was

repeated 5 times with intervals of 2 seconds. When 3 positive responses were observed, application of the filament was discontinued and this determined the mechanical pain threshold. A positive response was considered when paw withdrawal, paw licking, or sudden flinching could be observed. Ten animals were tested at the time and each filament was applied to each animal before using the next filament until pain threshold was reached. The test was conducted by two observers that were blind to mice phenotypes as cages were covered and experimenters could only see the hind paws. To make sure that withdrawal thresholds were determined correctly, both observers had to agree on withdrawal.

Acetone drop test

The acetone test was conducted to assess cold pain thresholds as previously described (Burke, et al., 2013). Animals were placed in transparent plastic cages with an elevated mesh floor and allowed 30 minutes of habituation. Polyethylene tubing (2 mm) was attached to 1 ml syringe and used to apply 0.02 ml of acetone (ACS reagent, ≥99.5%, Sigma-Aldrich, Canada) to the plantar surface of the hind paw. Response latencies were recorded for each animal within 60 seconds. A positive response was considered when paw withdrawal, paw licking, or sudden flinching could be observed. Each hind paw was tested 3 times with 3-minute intervals. If the tested animal did not show positive response within 60 seconds, this value was taken as the latency. The test was conducted by two experimenters who had to agree on the response latency.

Hot water immersion tail-flick test

The water immersion tail-flick test was conducted to assess hot pain thresholds as previously described (Elhabazi, et al., 2014). Mice were restrained in a Plexiglas restrainer. Two thirds of the distal part of each animal's tail was immersed in circulating hot water kept at 48°C. The latency at which the animal withdrew its tail from the hot water was recorded. Each mouse was tested 3 times with a 5-minute interval. Positive responses were usually observed within the first 5 seconds. The test was conducted by two experimenters who had to agree on the response latency.

Formalin test

Twenty blind mice and 20 sighted mice were injected with formalin. The formalin test was used to measure pain behaviors induced by a chemical stimulus. The test was video-recorded and performed by two experimenters who had to agree on the response occurrence. The formalin injection results in a two-phase reaction: the first phase, which is a neurogenic reaction, starts immediately after the injection and lasts around 10 minutes. The second phase, which is an inflammatory reaction, is observable in the 20-40 minutes time interval following the formalin injection (Barrot, 2012). Mice were individually exposed to the test chamber for 1 hour before the formalin injection. Using a 26-gauge needle, 25 µl of 5% formalin in a saline solution was injected subcutaneously into the plantar surface of the right hind paw. Animals were then placed in Plexiglas cages and were observed by two observers for a total of 45 minutes. The pain score was determined by calculating the number of paw lickings in five-minute intervals. Mice used as controls to assess baseline c-Fos immunoreactivity received no stimulation. They were taken from their cage and immediately perfused.

After the experiment, mice were anesthetized and perfused through the heart with 30 ml PBS solution (Phosphate Buffered Saline 0.1 M) mixed with 120 µL of contrast agent (Magnevist®: gadopentetate dimeglumine, Bayer Healthcare, Whippany, NJ, USA) followed by 30 ml of 4% paraformaldehyde solution (formaldehyde solution 37 wt.% in water, Sigma Aldrich, Missouri, USA) mixed with 120µL of the contrast agent at a flow rate of 1.0 ml/min. Immediately after perfusion, the head was removed and placed in a 4 % paraformaldehyde solution mixed with the contrast agent Magnevist at 4°C overnight. The contrast agent was used to improve the quality of magnetic resonance images but these images were not used for the present study.

C-Fos immunohistochemistry

Brains were collected and post-fixed in formalin for 24 hours and cryoprotected in a 30 % sucrose solution. Coronal sections of 50 µm thickness were collected using a freezing

microtome (Leica VT1000S, Leica microsystems, Wetzlar, Germany). For c-Fos protein immunohistochemistry, the sections were processed with the following steps: (1) washed with 0.1 M PBS 3 times (10 minutes each); (2) incubated for 30 minutes in hydrogen peroxide (H2O2) 3% in 0.1 M PBS; (3) washed 3 times in PBS (10 minutes each); (4) incubated in a PBS 0.1% Triton –X100 solution with normal donkey serum (2%) for 1 hour; (5) incubated overnight at 4 °C with a mouse monoclonal antibody (Ab208942) against the c-Fos protein (1:1500) in PBS 0.1% Triton –X100 with normal donkey serum (0.25%); (6) rinsed 3 times in PBS (10 minutes each); (7) incubated for 1 hour at room temperature with biotinylated preadsorbed donkey anti-mouse antibodies (Ab7060) (1:200) in PBS v0.1% Triton –X100 with normal donkey serum (0.25%); (8) rinsed 3 times in PBS (10 minutes each); (9) incubated for 1 hour in an avidin-biotin-peroxidase complex (1:250) in PBS v0.1% Triton –X100 solution; (10) rinsed 3 times in PBS (10 minutes each); (10) rinsed 2 times in a Tris Buffer solution (10 minutes each); (11) incubated in a solution of 0.02% 3.3-diaminobenzidine tetrahydrochloride, 0.04% nickel ammonium sulfate and 0.01% hydrogen peroxide in 0.05 M Tris-buffer (pH 7.6) for approximately 10 minutes at room temperature; (12) the reaction was stopped by washing the sections in PBS for 30 minutes and rinsing in distilled water when staining intensity was optimal (controlled under light microscope). Sections were then mounted on gelatin-coated slides, dried, and processed for Nissl staining, dehydrated, and cover-slipped. Sections were analyzed under an Olympus BX50W1 microscope coupled to a CCD camera (Optronix, MicroBrightField, Williston, VT, U.S.A.). C-Fos cell counting was done manually using the Neurolucida Software (MicroBrightField, Williston, VT, U.S.A.) and based on the Paxinos and Watson (1998) atlas (Paxinos and Franklin, 1998). C-Fos positive neurons were counted in the amygdaloid complex and in subnuclei, including the central (CeA) and basolateral (BLA/LA) amygdala. To determine if group differences may or may not be generalized to the entire brain, c-Fos positive neurons were also counted in the anterior cingulate cortex (ACC), an area involved in pain processing, and in the retrosplenial cortex (RSC), an area presumably not involved in pain processing.

Histological measurements

The volumes of the amygdaloid complex, CeA, BLA, ACC and RSC were measured on Nissl-stained brain sections under an Olympus BX50W1 microscope coupled to a CCD camera

(Optronix, MicroBrightField, Williston, VT, U.S.A.). Delineation of the amygdaloid complex, CeA, BLA, ACC and RSC was performed with the Neurolucida Software (MicroBrightField, Williston, VT, U.S.A.). Delineations were based on stereotaxic coordinates of Paxinos and Watson (1998) and on the cytoarchitectural description of amygdala nuclei in the rat (McDonald, 1982).

A total of twenty slices was analyzed. The contour of the structure was delineated on every second 50μm from Bregma -0.58 to Bregma -2.46 and the measured surface was multiplied by the distance between the slices (100 μm) then summed to obtain the total volume of each amygdala.

Statistical analyses

Statistical analyses were performed with Statistica v13.0 (Dell Inc., Tulsa, OK, USA). All data are expressed as mean ± S.E.M. Values of $p \leq 0.05$ were considered statistically significant. Mechanical and thermal pain thresholds, pain behaviors in the formalin test, c-Fos immunoreactivity as well as structure volumes were compared with mixed ANOVAs (see details for each analysis in the results section). Effect sizes are reported based on partial eta-squared (η2p).

Results

Pain sensitivity assessment

Mechanical hyperalgesia in the anophthalmic mouse

The mechanical pain thresholds for the right and left hind paws were compared between anophthalmic and sighted mice with a mixed ANOVA (see Figure 1A). The hind paw withdrawal threshold was lower in anophthalmic compared with sighted mice (main effect: $F_{1,16}=19.3$, $p<0.001$, $\eta^2_p=0.55$). This effect was not significantly different between paws (interaction: $F_{1,16}=0.16$, $p=0.7$, $\eta^2_p<0.1$) or between males and females (interaction: $F_{1,16}=1.6$, $p=0.2$, $\eta^2_p<0.09$).

Thermal hyperalgesia in the anophthalmic mouse

Acetone drop test

The mean cold pain thresholds for both hind paws were compared between anophthalmic and sighted mice with a mixed ANOVA (see Figure 1B). Thresholds were lower in anophthalmic compared with sighted mice (main effect: $F_{1,16}=7.4$, $p=0.015$, $\eta^2_p=0.31$). This effect was not significantly different between paws (interaction: $F_{1,16}=0.01$, $p=0.9$, $\eta^2_p<0.001$) or between males and females (interaction: $F_{1,16}=0.01$, $p=0.9$, $\eta^2_p<0.001$).

Water immersion Tail-Flick test

The mean heat pain thresholds were compared between anophthalmic and sighted mice with a two-way ANOVA (see Figure 1C). Thresholds were lower in anophthalmic compared with sighted mice (main effect: $F_{1,16}=20.1$, $p<0.001$, $\eta^2_p=0.56$). This effect was not significantly different between males and females (interaction: $F_{1,16}=0.4$, $p=0.5$, $\eta^2_p=0.02$).

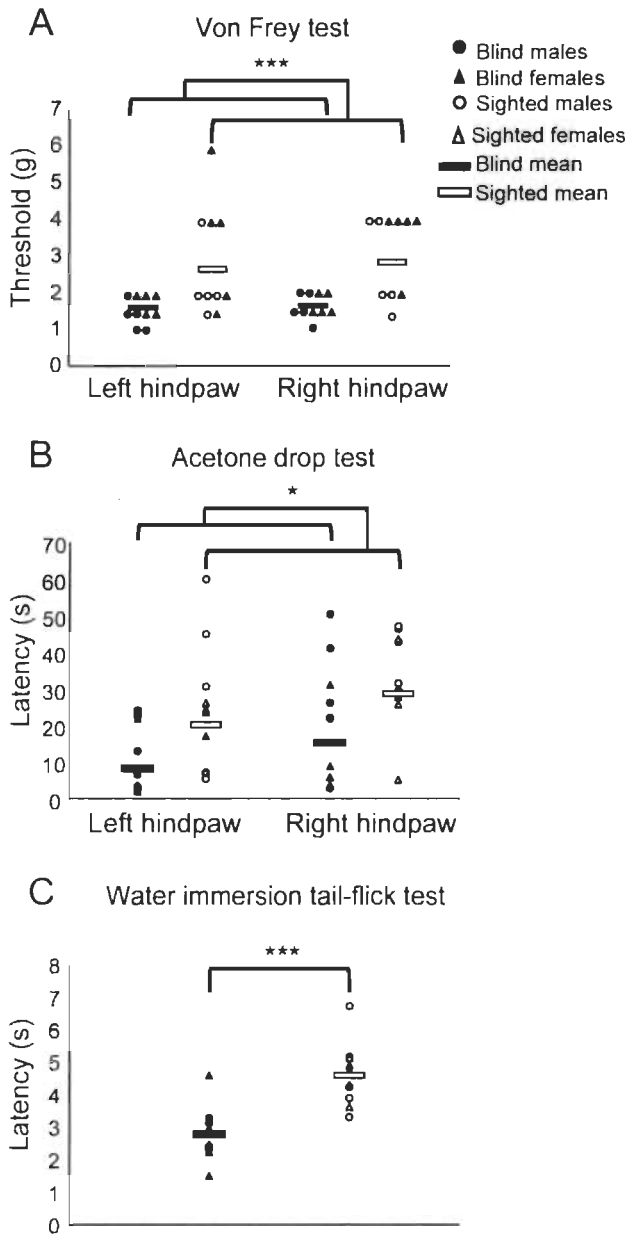

Figure 3-1: Hyperalgesia in the blind mouse

A. von Frey test: The hind paw withdrawal threshold was lower in anophthalmic compared with sighted mice ($p<0.001$). This effect was not significantly different between paws ($p=0.7$) or between males and females ($p=0.2$). ***B****. Acetone drop test: Latencies were lower in blind compared with sighted mice indicating cold allodynia ($p=0.015$). The mean latency was 32.5 s and 24.7 s in sighted mice versus 20.9 s and 12.3 s in blind mice for the right and the left hind paw, respectively. There was no significant difference between paws ($p=0.9$) or between* males

*and females p=0.9). **C**. Hot water immersion Tail-Flick test: Latencies for tail withdrawal from hot water were lower in blind compared with sighted mice, indicating thermal hyperalgesia (p<0.001). The mean latency was 4.6 s in sighted mice versus 2.7 s in blind mice. There was no significant difference between sexes (p=0.5). Sample details are presented in Table 1.*P < 0.05; ***P < 0.001.*

Increased pain behaviors in the anophthalmic mouse

Formalin injection in the right hind paw produced time dependent pain behaviors in both groups as evidenced by licking behaviors in acute and inflammatory phases. Pain scores were compared between groups across phases with a mixed ANOVA (see Figure 2). Pain scores were different between groups across phases (interaction: $F_{1,10}$=8.9, p=0.01; η^2_p=0.47), but this effect was not significantly different between males and females (interaction: $F_{1,10}$=3.7, p=0.08; η^2_p=0.27). The Fisher posthoc test revealed that formalin produced more pain behaviors in anophthalmic compared with sighted mice in the acute phase (p<0.001), but not in the inflammatory phase (p=0.7).

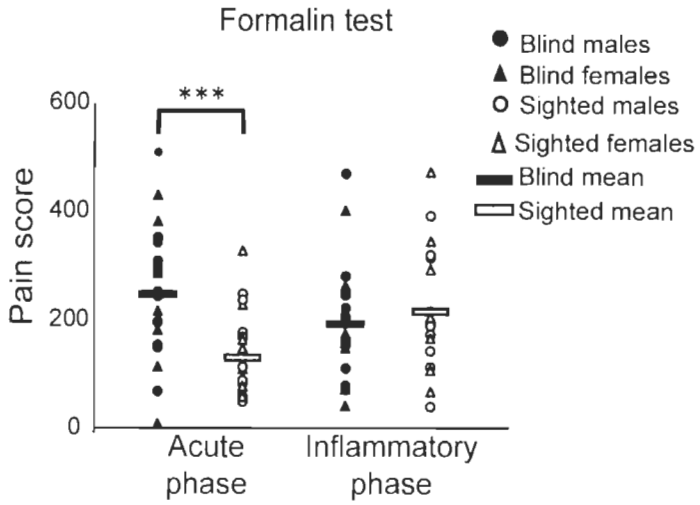

Figure 3-2 : Increased pain behaviors in the blind mouse

The pain score corresponding to the number of paw lickings was counted during the acute and inflammatory phases. Pain scores were different between groups across phases (p=0.01). The Fisher posthoc test revealed that formalin produced more pain behaviors in blind compared

*with sighted mice in the acute phase ($p<0.001$) but not in the inflammatory phase ($p=0.7$). Sample details are presented in Table 1. ***P <0 .001.*

Stronger pain-related activity in the amygdala of the anophthalmic mouse

The number of c-Fos immunoreactive neurons within the amygdaloid complex as well as its central nucleus (CeA) and basolateral/lateral nuclei (BLA/LA) were compared between groups, hemispheres and conditions (formalin vs control) with mixed ANOVAs (see Figure 3). Individual examples from each group showing anatomical delimitations and c-Fos immunoreactive neurons are shown in Figure 4.

For the amygdaloid complex, the number of c-Fos immunoreactive neurons was significantly different between groups across conditions (formalin vs control) (interaction: $F_{1,16}= 12.3$, $p=0.003$; $\eta^2_p=0.44$). This effect was not significantly different between hemispheres (interaction: $F_{1,16}=0.7$, $p=0.4$; $\eta^2_p=0.04$) or between males and females (interaction: $F_{1,16}=0.04$, $p=0.9$; $\eta^2_p<0.01$). Planned contrasts revealed that the number of c-Fos positive neurons was significantly greater in anophthalmic compared with sighted mice in the fomalin vs control condition for both the right ($p=0.003$) and left ($p=0.005$) amygdaloid complex. Moreover, anophthalmic mice showed increased c-Fos positive neurons in the formalin compared with the control condition for both the left ($p<0.001$) and right ($p<0.001$) hemispheres, while this effect was not significant in sighted mice for either the right or left hemispheres ($p=0.22$ and $p=0.44$, respectively). In addition, no significant difference was observed between sighted and anophthalmic mice in the control condition for the right ($p=0.9$) or left ($p=0.9$) hemisphere, indicating that the group difference was not due to differences in baseline activity.

For the CeA, the number of c-Fos immunoreactive neurons was significantly different between groups across conditions (formalin vs control) (interaction: $F_{1,16}=16.5$, $p<0.001$; $\eta^2_p=0.51$). This effect was not significantly different between hemispheres (interaction: $F_{1,16}=0.1$, $p=0.7$; $\eta^2_p<0.01$) or between males and females (interaction: $F_{1,16}= 2.5$, $p=0.14$; $\eta^2_p=0.13$). Planned contrasts revealed that the number of c-Fos positive neurons was significantly greater in anophthalmic compared with sighted mice in the fomalin vs control condition for both the right ($p<0.001$) and left ($p=0.01$) CeA. Moreover, anophthalmic mice showed increased c-Fos

positive neurons in the formalin compared with the control condition for both the left ($p<0.001$) and right ($p<0.001$) hemispheres, while this effect was not significant in sighted mice for either the right or left hemispheres ($p=0.062$ and $p=0.30$, respectively). In addition, no significant difference was observed between sighted and anophthalmic mice in the control condition for the right ($p=0.9$) or left ($p=0.9$) hemisphere, indicating that the group difference was not due to differences in baseline activity.

For the BLA/LA, the number of c-Fos immunoreactive neurons was not significantly different between groups across conditions (formalin vs control) (interaction: $F_{1,16}=0.3$, $p=0.6$; $\eta^2_p=0.02$). This effect was also not significantly different between hemispheres (interaction: $F_{1,16}=0.9$, $p=0.4$; $\eta^2_p=0.05$) or between males and females (interaction: $F_{1,16}=0.003$, $p=0.96$; $\eta^2_p<0.01$).

To determine whether stronger formalin-induced c-Fos expression could be observed in other brain regions of anophalmic compared with sighted mice, the ACC was examined as a pain-related region and the RSC as a region presumably not involved in pain (see Figure 5).

For the ACC (Figure 5A), the number of c-Fos positive neurons was not significantly different between groups (main effect: $F_{1,10}=0.18$, $p=0.2$; $\eta^2_p=0.01$), between groups and sexes (interaction: $F_{1,10}=2.7$, $p=0.13$; $\eta^2_p=0.21$) or between groups and hemispheres (interaction: $F_{1,10}=1.3$, $p=0.3$; $\eta^2_p=0.12$).

For the RSC (Figure 5B), the number of c-Fos positive neurons was not significantly different between groups (main effect: $F_{1,10}=0.11$, $p=0.7$; $\eta\eta^2_p=0.01$) or between groups and sexes (interaction: $F_{1,10}=0.4$, $p=0.5$; $\eta^2_p=0.02$) as well as between groups and hemispheres (interaction: $F_{1,10}=0.01$, $p=0.99$; $\eta^2_p<0.01$).

To examine whether pain behaviors during the acute phase of the formalin test could predict the number of c-Fos immunoreactive neurons, regression analyses were performed for both groups combined. Higher pain scores during the acute phase predicted stronger c-Fos immunoreactivity in the right and left amygdaloid complex (both $R^2=0.50$, $p=0.005$) as well as in the right and left CeA (both $R^2=0.36$, $p=0.02$) (see Figure 6). This was not the case for the right and left BLA/LA ($R^2=0.08$, $p=0.3$ and $R^2=0.14$, $p=0.2$, respectively), the right and left

ACC (R^2=0.03, p=0.6 and R^2=0.01, p=0.7, respectively) and the right and left RSC (R^2=0.3, p=0.3 and R^2=0.04, p=0.5, respectively).

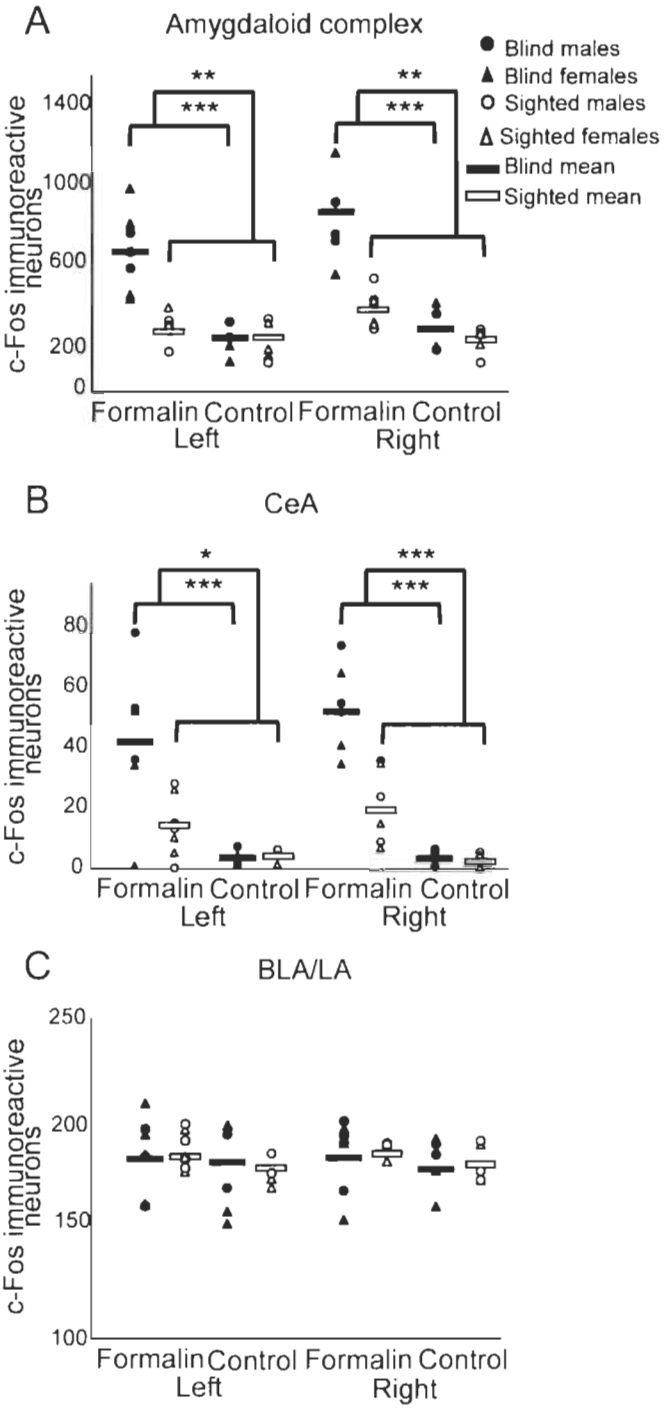

Figure 3-3: Increased pain-related activity in the amygdala and CeA of the blind mouse

A. *The number of c-Fos immunoreactive neurons was significantly different between groups across conditions (formalin vs control) ($p=0.003$). This effect was not significantly different between hemispheres ($p=0.4$) or between males and females ($p=0.9$). The number of c-Fos positive neurons was significantly greater in anophthalmic compared with sighted mice in the fomalin vs control condition for both the right ($p=0.003$) and left ($p=0.005$) amygdaloid complex. Anophthalmic mice showed increased c-Fos positive neurons in the formalin compared with the control condition for both the left ($p<0.001$) and right ($p<0.001$) hemisphere. This effect was not significant in sighted mice for either the right or left hemispheres ($p=0.22$ and $p=0.44$, respectively). No significant difference was observed between sighted and anophthalmic mice in the control condition for the right ($p=0.9$) or left ($p=0.9$)*

B. *For the CeA, the number of c-Fos immunoreactive neurons was significantly different between groups across conditions (formalin vs control) ($p<0.001$). This effect was not significantly different between hemispheres ($p=0.7$) or between males and females ($p=0.14$). The number of c-Fos positive neurons was significantly greater in anophthalmic compared with sighted mice in the fomalin vs control condition for both the right ($p<0.001$) and left ($p=0.01$) CeA. Anophthalmic mice showed increased c-Fos positive neurons in the formalin compared with the control condition for both the left ($p<0.001$) and right ($p<0.001$) hemispheres. This effect was not significant in sighted mice for either the right or left hemispheres ($p=0.062$ and $p=0.30$, respectively). No significant difference was observed between sighted and anophthalmic mice in the control condition for the right ($p=0.9$) or left ($p=0.9$) hemisphere.*

C. *For the BLA/LA, the number of c-Fos immunoreactive neurons was not significantly different between groups across conditions (formalin vs control) ($p=0.6$). This effect was also not significantly different between hemispheres ($p=0.4$) or between males and females (=0.003, $p=0.96$). Sample details are presented in Table 1.*P < 0.05; **P <0 .01;***P < 0.001.*

Larger amygdala volume in the anophthalmic mouse

The amygdaloid complex, the CeA, the BLA/LA, as well as the ACC and the RSC were delineated based on histological features and stereotaxic coordinates. The volumes of each structure were compared between groups, sexes and hemispheres with mixed ANOVAs (see Figure 7 and 8).

The amygdaloid complex volume was larger in anophthalmic compared with sighted mice (main effect: $F_{1,20}$=10.3, p=0.004; η^2_p=0.34). This effect was not significantly different between hemispheres (interaction: $F_{1,20}$ =2.1, p=0.17; η^2_p=0.09) or between males and females (interaction: $F_{1,20}$ =2.5, p=0.13; η^2_p=0.11) (see Figure 7A).

For the CeA, no significant volume difference was observed between groups (main effect: $F_{1,20}$=0.2, p=0.7; η^2_p=0.01), between groups across hemispheres (interaction: $F_{1,20}$=0.2, p=0.9; η^2_p<0.001) or between groups across sexes (interaction: $F_{1,20}$=0.8, p=0.4; η^2_p=0.04) (see Figure 7B).

For the BLA/LA, no significant volume difference was observed between groups (main effect: $F_{1,20}$=0.7, p=0.4; η^2_p=0.03), between groups across hemispheres (interaction: $F_{1,20}$=0.1, p=0.7; η^2_p=0.005) or between groups across sexes (interaction: $F_{1,20}$=0.2, p=0.7; η^2_p=0.009) (see Figure 7C).

For the ACC, no significant volume difference was observed between groups (main effect: $F_{1,10}$=0.1, p=0.8; η^2_p=0.01), between groups across hemispheres (interaction: $F_{1,10}$=0.1, p=0.8; η^2_p=0.01) or between groups across sexes (interaction: $F_{1,10}$=0.4, p=0.5; η^2_p=0.04) (see Figure 8A).

Finally, for the RSC, no significant volume difference was observed between groups (main effect: $F_{1,10}$=0.1, p=0.8; η^2_p=0.01), between groups across hemispheres (interaction: $F_{1,10}$=0.1, p=0.8; η^2_p=0.01) or between groups across sexes (interaction: $F_{1,10}$=1.6, p=0.2; η^2_p=0.15) (see Figure 8B).

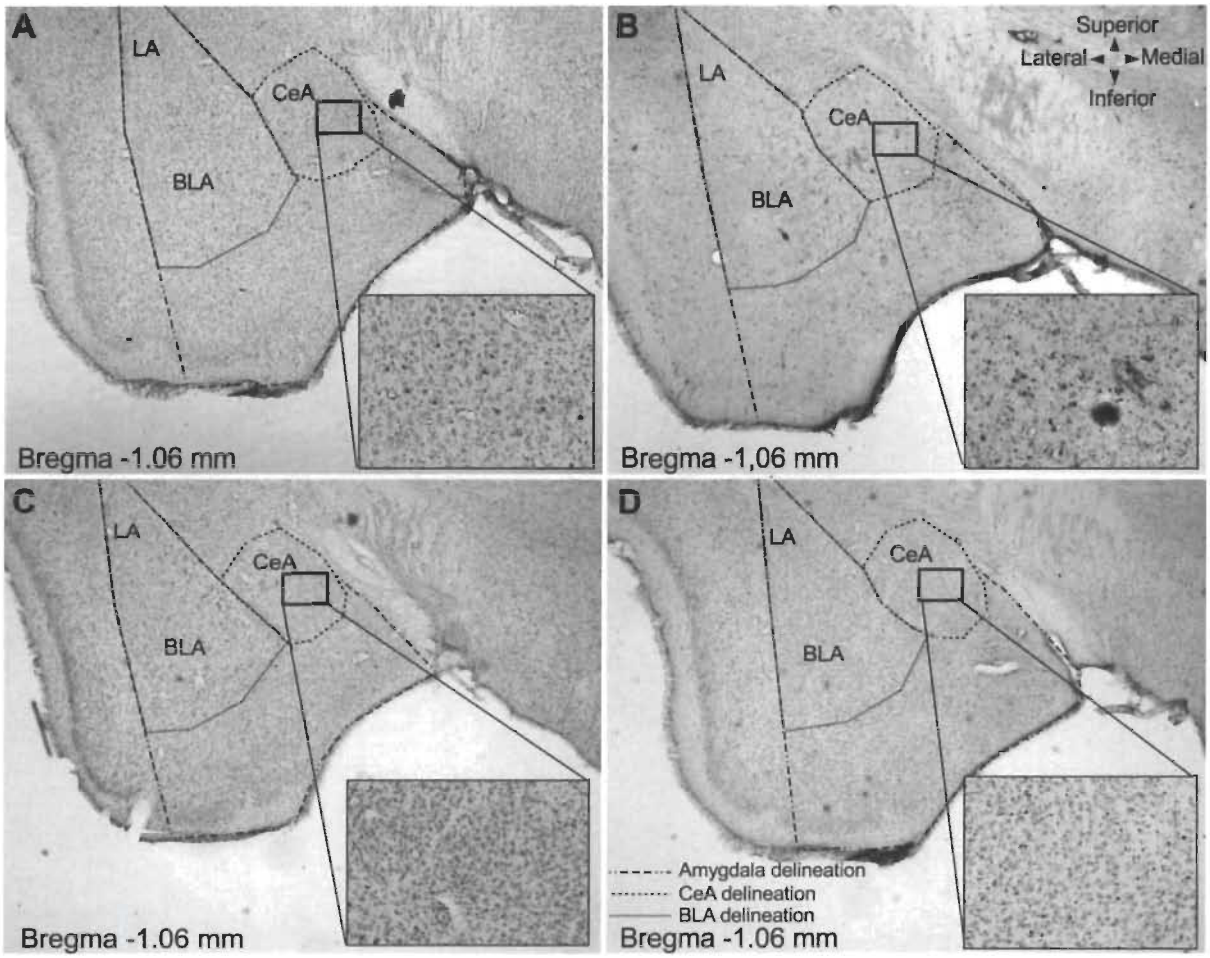

Figure 3-4: Delineation of the amygdaloid complex, CeA, BLA/LA and c-Fos immunoreactivity

Photomicrographs of the delineation of the amygdaloid complex and of the central amygdaloid nucleus (CeA) in the right hemisphere on Nissl stained and c-Fos immunoreacted coronal sections. ***A****. Coronal brain section of the right hemisphere at Bregma −1.06 mm in a sighted mouse with formalin injection.* ***B****. Coronal brain section of the right hemisphere at Bregma −1.06 mm in a blind mouse with formalin injection.* ***C****. Coronal brain section of the right hemisphere at Bregma −1.06 mm in a sighted control mouse.* ***D****. Coronal brain section of the right hemisphere at Bregma −1.06 mm in a blind control mouse. The number on c-Fos immunoreactive neurons was greater in anophthalmic compared with sighted mice (B and D vs*

A and C) and greater in the formalin compared with control condition (A and B vs C and D). Statistical analyses for group comparisons are presented in Fig. 3.

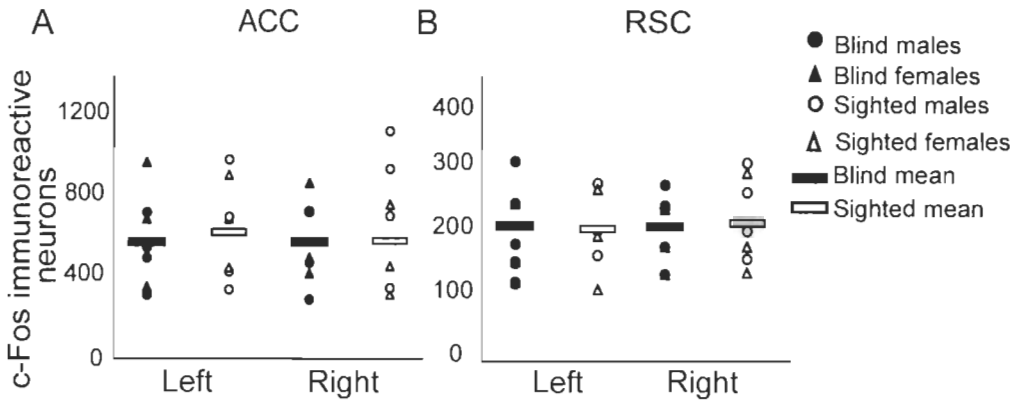

Figure 3-5: c-Fos immunoreactivity in the ACC and RSC

A*. For ACC, the number of c-Fos positive neurons was not significantly different between groups (P=0.2), between groups and sexes (P=0.13) or between groups and hemispheres (P=0.3).* ***B****. For RSC, the number of c-Fos positive neurons was not significantly different between groups (P=0 .7), between groups and sexes (P=0.5) or between groups and hemispheres (P=0.99). Sample details are presented in Table 1.*

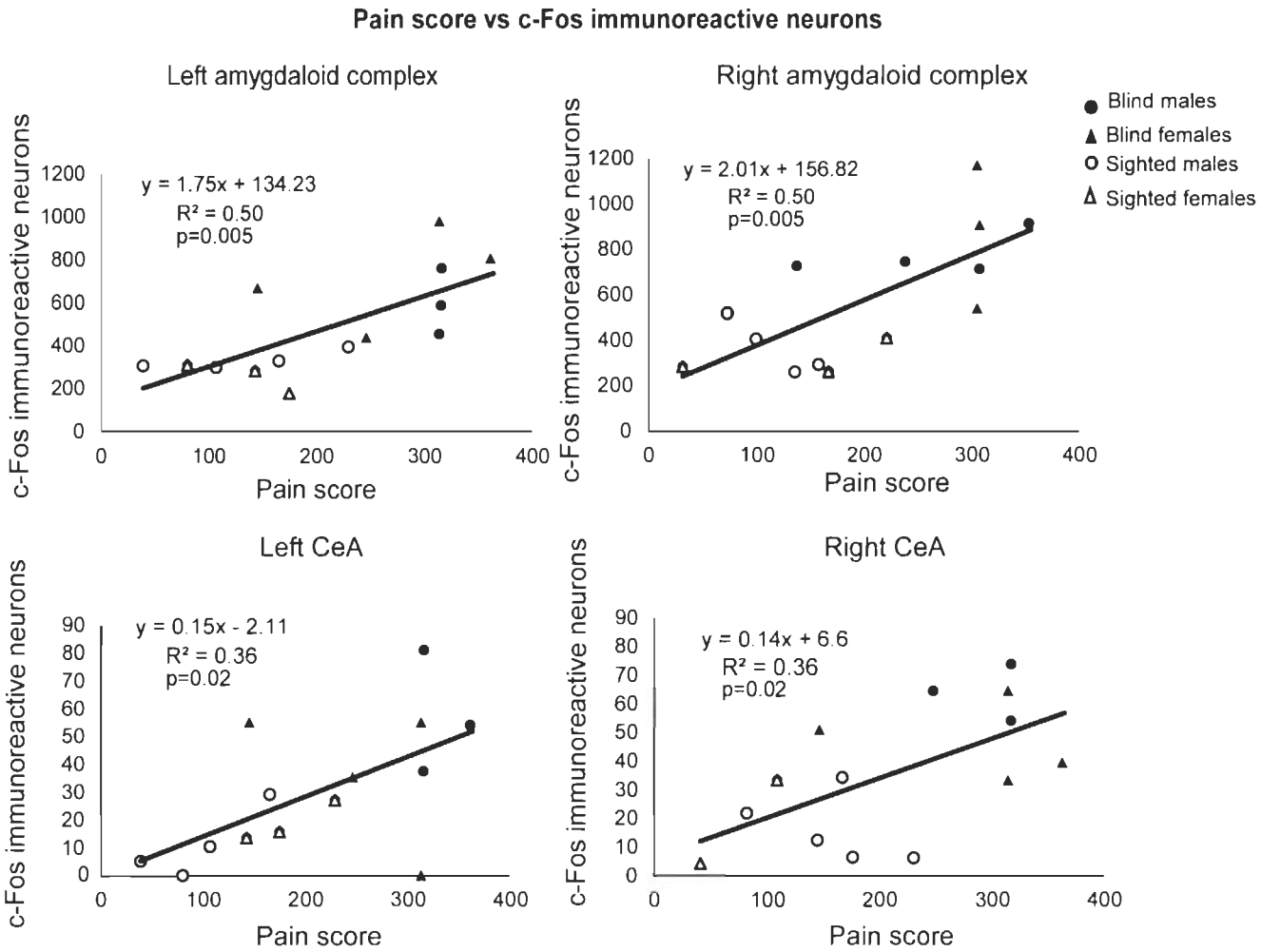

Figure 3-6: Prediction of c-Fos immunoreactive neurons by pain behaviors in formalin test

Higher pain scores during the acute phase of the formalin test predicted stronger c-Fos immunoreactivity in the right and left amygdaloid complex (both R2 = 0.50, P = 0.005) as well as in the right and left CeA (both R2 = 0.36, P = 0.02).

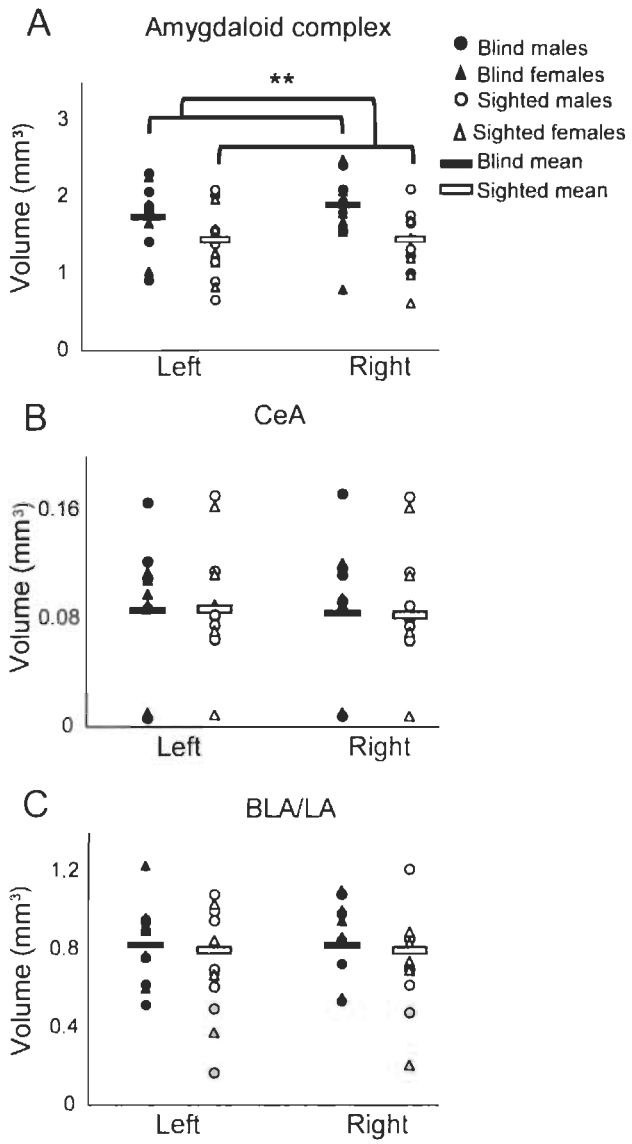

Figure 3-7: Increased amygdala volume in the anophthalmic mouse

A. The amygdaloid complex volume was larger in anophthalmic compared with sighted mice (P = 0.004), but this effect was not significantly different between groups across hemispheres (P =0 .17) or between groups across sexes (P = .13). ***B****. For the CeA, no volume difference was observed between groups (P = 0.7), between groups across hemispheres (P = 0.9) or between groups across sexes (P = 0.4).* ***C****. For the BLA/LA, no volume difference was observed between groups (P =0.4), between groups across hemispheres (P = 0.7) or between groups across sexes (P = 0.7). Sample details are presented in Table 1. **P < 0.01.*

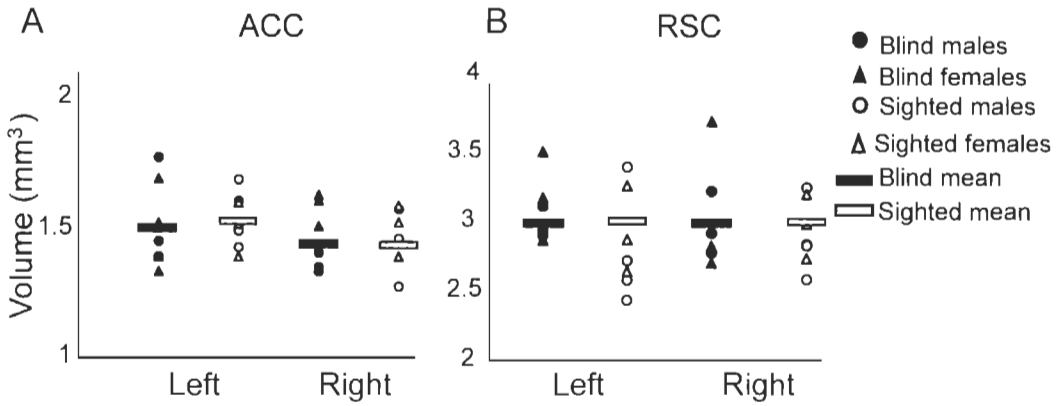

Figure 3-8: ACC and RSC volumes

A. For the ACC, no volume difference was observed between groups (P = 0.8), between groups across hemispheres (P = 0.8) or between groups across sexes (P = 0.5). ***B****. For the RSC, no volume difference was observed between groups (P = 0.8), between groups across hemispheres (P = 0.8) or between groups across sexes (P =0.2). Sample details are presented in Table 1.*

Discussion

The present study provides a mouse model of congenital blindness in which blind mice can be compared with sighted mice with the same genetic background. This is the first animal study on pain and blindness. The novel findings of the present study are that mechanical and thermal pain thresholds are decreased and pain behaviors during the formalin test are increased in blind compared with sighted mice. Moreover, increased pain behaviors during the formalin test predicted the increased pain-related activity in the amygdala observed in blind mice. Furthermore, amygdala volume was increased in blind compared with sighted mice. These results indicate that congenitally blind mice show pain hypersensitivity similar to that observed in early blind individuals and suggest that this is due, in part, to plasticity in the amygdala.

Congenital blindness and pain hypersensitivity

The present results indicate that congenitally blind mice have lower mechanical and thermal pain thresholds and increased pain sensitivity to a noxious chemical stimulus compared

with sighted mice. This is consistent with human studies on pain and visual deprivation or blindness. The first study showing the impact of visual deprivation on pain perception was conducted in 1964 (Zubek, et al., 1964). In that study, Zubek et al. showed that blindfolding normally sighted individuals for 7 days resulted in increased heat pain sensitivity to acute stimuli. This enhanced pain perception persisted for several days after the experiment, suggesting that long-lasting changes occurred due to visual deprivation (Zubek, et al., 1964). In accordance with these findings, lower heat and cold pain thresholds as well as increased perception of suprathreshold nociceptive stimuli were reported in early blind individuals compared with controls with normal vision (Slimani, et al., 2013). These differences were not observed in late-onset blindness (Slimani, et al., 2014), suggesting that there is a critical period during which enhanced pain sensitivity develops. However, this contrasts with results obtained from blindfolding in normally sighted individuals. Future studies are needed to determine the mechanisms of pain hypersensitivity in blindness and during transient visual deprivation.

Plasticity in the amygdala as a mechanism of pain hypersensitivity in the blind

C-Fos has been widely used as a marker of neuronal activity (Bullitt, 1990; Martinez, et al., 2002). Several studies showed that different types of noxious stimuli can induce c-Fos expression in specific areas of the brain and spinal cord (Bullitt, 1990; Chan, et al., 1993; Harris, 1998). In the present study, formalin injection in the right hind paw induced c-Fos expression in different subnuclei of the amygdala, including the CeA. This activation was significantly higher than baseline activity in control mice (no formalin injection). The quantitative analysis of c-Fos expression showed stronger pain-related activity in the amygdaloid complex and in the CeA more specifically, in anophthalmic compared with sighted mice. This effect was not observed for the BLA/LA. Moreover, formalin-induced c-Fos expression was not significantly different between anophthalmic and sighted mice for ACC and RSC. The lack of group difference for BLA and ACC suggests that increased pain-related activity in anophthalmic mice is not generalized to all pain-related structures. The lack of group difference for RSC, a region presumably not involved in pain processing, also suggests that increased c-Fos expression in CeA is not simply due to a generalized increase in c-Fos expression in the brain of anophthalmic mice. In line with these interpretations, pain scores in the acute phase of the formalin test

predicted c-Fos immunoreactivity in the amygdaloid complex and the CeA, but not in BLA/LA, ACC or RSC. This suggests that the amygdala and more specifically the CeA contribute to pain hypersensitivity in the anophthalmic mouse. This does not exclude that other brain regions that were not examined may contribute to pain hypersensitivity in blind mice. These results are consistent with previous studies reporting c-Fos protein expression in the amygdala in response to a variety of noxious stimuli including formalin (Traub, et al., 1996; Nakagawa, et al., 2003; Suwanprathes, et al., 2003; Lanuza, et al., 2008; Miyazawa, et al., 2018; Seno, et al., 2018). Results are also consistent with the key role of the amygdala in emotions, pain processing, pain modulation, and chronic pain (Fanselow and LeDoux, 1999; Fanselow and Gale, 2003; Phelps and LeDoux, 2005; Veinante, et al., 2013; Neugebauer, 2015; Kato, et al., 2018; Miyazawa, et al., 2018). Indeed, behavioral and physiological studies have shown functional changes in the amygdala in different pain conditions and pain models (Bernard, et al., 1992; Bornhovd, et al., 2002; Berman, et al., 2006). Although plasticity induced by congenital blindness and by chronic pain in the amygdala seems to be based on different mechanisms, both models lead to increased pain-related activity in the amygdala and widespread hyperalgesia. Lateralization of the CeA activation following painful stimuli has been observed in different studies (Sadler, et al., 2017; Cooper, et al., 2018; Miyazawa, et al., 2018). In the case of an inflammatory pain model, regardless of the side injected with formalin, it was shown that the right CeA was predominantly activated by pain in spite of the bilateral activation of the lateral parabrachial nucleus (Miyazawa, et al., 2018). In the case of blind animals in the present study, higher pain scores during the formalin test were associated with stronger c-Fos immunoreactivity in both CeA. Therefore, involvement of both CeA in blind mice might be due to plasticity induced by blindness. Sustained or chronic pain seems to depend on the right CeA, while higher pain sensitivity due to blindness involves both CeA. Future studies are needed to understand the mechanisms that link pain hypersensitivity to bilateral activation of the amygdala in blind mice.

More generally, the present findings are consistent with the role of the amygdala and CeA in pain perception and modulation. For instance, the bilateral resection of the amygdala, hippocampus, and parahippocampal gyrus resulted in a drastic reduction of pain sensation in a patient undergoing neurosurgery. This pain sensation reduction was likely due to the amygdala resection, since all other pain-related structures remained intact (Hebben, et al., 1985). Also, it

was reported that the blockade of Extracellular signal-regulated Kinase (ERKs) in the amygdala reduces tactile hypersensitivity and that pharmacological activation of ERKs in the amygdala induces tactile hypersensitivity (Carrasquillo and Gereau, 2007). In other studies, the injection of antinociceptive neuropeptides in the CeA produced analgesia (Ahn, et al., 2001; Ortiz, et al., 2007) and reduced thermal and mechanical hypersensitivity (Oliveira and Prado, 1994; Han and Neugebauer, 2005). Moreover, the inactivation or lesioning of CeA drastically reduces opioid-induced analgesia (Manning and Mayer, 1995; Manning, et al., 2003). Furthermore, activation of anti-nociceptive neuropeptides in CeA in a neuropathic pain model reduces mechanical pain hypersensitivity (Pedersen, et al., 2007), while activation of pro-nociceptive ERKs in CeA produces sustained mechanical hypersensitivity in an arthritis model (Fu, et al., 2008). These results support the important contribution of the amygdala and CeA to pain modulation and support a role for the amygdala in pain hypersensitivity of blind mice observed in the present study. This does not exclude the contribution of other pain-related areas that may interact with the amygdala or may contribute to pain hypersensitivity in the blind independently of the amygdala.

Structural plasticity of the amygdala in the blind mouse

A larger amygdaloid complex was observed in anophthalmic compared with sighted mice, while no volumetric difference was observed between groups for CeA, BLA/LA, ACC and RSC. The lack of volumetric difference between groups for individual subnuclei of the amygdala suggests that cumulative volumetric differences in several amygdaloid subnuclei could contribute to the group difference. Alternatively, the volumetric analysis may not be sensitive enough to allow the detection of group differences for small subnuclei. Nonetheless, these results are consistent with bilateral amygdala volume increase in rats with pain hypersensitivity and neuropathic pain (Goncalves, et al., 2008). Again, plasticity induced by blindness or chronic pain may be different. Accordingly, cerebral plasticity associated with behavioral changes is evidenced in some brain regions in early blind humans. A larger hippocampal volume and a better spatial navigation have been observed in early blind subjects (Fortin, et al., 2008). A larger olfactory bulb and a better olfactory function were also observed in early blind subjects (Rombaux, et al., 2010). The volume increase in the amygdala in blind

mice might be the result of intermodal plasticity after congenital blindness, which is likely different from synaptic potentiation that occurs in the amygdala in response to sustained pain. In the neuropathic pain model, it was shown that the volume increase in the amygdala was more likely due to an increased number of neurons (Goncalves, et al., 2008). Future studies are needed to investigate the exact mechanism leading to larger amygdala volume in blind mice.

Limitations and future directions

The ZRDBA mouse cross allows studying the impacts of early blindness on behavioral and neural changes. In humans, it was shown that only early blind, but not late-onset blind individuals exhibit pain hypersensitivity (Slimani, et al., 2014). It would be interesting to assess pain perception in a mouse model of late-onset blindness and compare the morphometry and functional activation of the amygdala and CeA in this model. To obtain a mouse model of late-onset blindness, enucleation could be performed in the same ZRDBA cross.

In this study, differences in brain structure and function were assessed in the amygdaloid complex and the CeA, to test the hypothesis of the role of the amygdala in pain hypersensitivity in blind mice as supported by previous studies on pain and the amygdala. This does not exclude that other brain regions involved in pain processing may have contributed to the present findings. A whole brain analysis of morphology and pain-related activity would be suitable to test this possibility, using MRI for instance.

Moreover, in order to establish a causal relationship between changes observed in the amygdala and pain hypersensitivity in the blind mouse, further manipulations are needed. A chemogenetic approach would be useful in this regard to alter neuronal activity in the amygdala in order to confirm that increased activity or decreased inhibition in the amygdala of the blind mouse causes pain hypersensitivity.

Furthermore, the amygdala is involved in several other functions including emotions and behavioral conditioning. It would be interesting to study the impact of the amygdala plasticity observed in this study on other physiological and behavioral features.

It has been previously shown in other studies that circadian rhythm may influence pain sensitivity (Göbel and Cordes, 1990; Konecka and Sroczynska, 1998; Bachmann, et al., 2011).

In the present study, sighted and blind animals were housed in mixed cages, which regulates blind mice circadian rhythm in accordance with that of sighted mice. In blind mice, circadian rhythms can be entrained by feeding times and other activities with littermates, as shown previously (Laemle and Ottenweller, 1999). Potential confounding effects on pain sensitivity, are unlikely since animals were tested together and at the same time, such that small difference in circadian rhythms, if any, could not explain group differences in pain sensitivity (Bachmann, et al., 2011). Nevertheless, it has been shown that the circadian rhythm of blind individuals is slightly lengthened (about 25 instead of 24 hours) (Lewy, et al., 1992). Thus, a slight daily phase shift eventually leads to an important phase shift in 24 days in blind individuals. This may affect pain sensitivity. However, it is unlikely that this effect explains the present results as in this case, circadian rhythms are not synchronized, vary randomly, and fluctuations can either increase or decrease pain sensitivity individually. Importantly, the tail-flick and acetone tests were conducted in the same animals on the same day, but the former in the morning and the latter in the afternoon. Considering that both tests produced the same results, it is assumed that results do not depend on circadian variations.

Lastly, the present study includes both male and female mice. This allows a better generalization of the results to both sexes. However, it is also a limitation of the present study because of the sample size and because of sex differences in pain perception that were reported previously (Mogil, et al., 2000; Wiesenfeld-Hallin, 2005; Mogil, 2009; Sorge and Totsch, 2017). Indeed, we observed that the effects of blindness on pain sensitivity and brain activity and structure were not significantly different between males and females. However, this does not imply pain perception and processing is not different between sexes. Future studies with larger sample size specifically designed to test sex differences are needed to confirm the present findings regarding sex differences.

Conclusion

In conclusion, the present study provides evidence that mechanical and thermal pain thresholds are decreased and pain behaviors during the formalin test are increased in blind compared with sighted mice. Moreover, higher pain scores during the formalin test predicted

the stronger pain-related activity in the amygdala observed in blind mice, suggesting a potential contribution of the amygdala in pain hypersensitivity due to blindness. This study also provides a novel mouse model of congenital blindness that is of high interest for further study of the mechanisms of pain hypersensitivity in the blind.

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Conflicts of interest

The authors declare no competing interests and no relationship that may lead to any conflict of interest.

Abbreviations

CeA: Central nucleus of the amygdala; BLA/LA: Basolateral nucleus of the amygdala; ACC: anterior cingulate cortex; RSC : Retrosplenial cortex; PBS: Phosphate Buffer Saline; ERK: Extracellular signal-regulated Kinase.

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Chapitre IV Article 3 - Structural brain plasticity induced by early blindness

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Abstract

It is well established that early blindness results in behavioral adaptations as well as functional and structural plasticity. While the behavioral and functional effects of visual deprivation have been well researched, anatomical studies are scarce. The aim of this study was to investigate structural brain plasticity in a mouse model of congenital blindness. Whole-brain volumetric analyses were conducted on high-resolution MRI images. In addition, complementary histological morphometric analyses were performed on slices from the same brains. These morphometric measurements were compared between anophthalmic and sighted mice issued from the ZRDBA strain, obtained by breeding ZRDCT and DBA mice. Results from MRI analyses using the Multiple

Automatically Generated Templates method showed smaller volume for the primary visual cortex and superior colliculi in anophthalmic compared with sighted mice. In addition, deformation-based morphometry revealed smaller volumes within the dorsal lateral geniculate nuclei and the lateral secondary visual cortex and larger volumes within olfactory areas, piriform cortex, orbital areas, and the amygdala, in anophthalmic compared with sighted mice. Histological analyses revealed a larger volume for the amygdala and smaller volume for the superior colliculi, primary visual cortex, and medial secondary visual cortex, in anophthalmic compared with sighted mice. Superior colliculus volume was smaller due to the absence of superficial visual layers, and the smaller primary visual cortex was due to decreased cortical layer IV thickness. Future investigations using this mouse model will allow determination of the underlying mechanisms of intramodal and crossmodal brain plasticity in visually deprived animals, as well as its behavioral consequences.

Introduction

The brain can adapt to the lack of sensory input when sensory organs are not present from birth or are lost during development or during adult life (Frasnelli et al.,2011). In the case of early blindness, functional and structural brain plasticity allows behavioral adaptation to visual deprivation (Kupers & Ptito, 2014). In humans, early blindness results in enhanced pitch discrimination (Gougoux et al., 2004; Hamilton et al.,2004; Wan et al., 2010), better sound localization (Lessard et al., 1998; Roder et al., 1999; Fieger et al., 2006), enhanced tactile acuity (Van Boven et al., 2000; Goldreich & Kanics, 2003; Beaulieu-Lefebvre et al., 2011; Wong et al., 2011), lower olfactory threshold (Cuevas et al., 2010; Comoglu et al., 2015), enhanced olfactory discrimination and identification (Smith et al., 1993; Cuevas et al., 2010; Rombaux et al., 2010; Renier et al., 2013), and increased pain sensitivity to cutaneous thermal stimuli (Slimani H, 2014; Slimani et al., 2015). In line with the brain abilities to adapt to sensory deprivation, several studies indicate that behavioral adaptations in the blind are generally supported by functional and structural brain plasticity (Kupers & Ptito, 2014).

In early blind animals, the occipital cortex may be activated by non-visual stimuli (Bonaventure and Karli, 1968; Heil, et al., 1991; Gao and Pallas, 1999; Ptito, 2001; Bronchti, et al., 2002; Piche, et al., 2004; Chabot, et al., 2007). Activation of the occipital cortex by non-visual tasks has also been shown in early blind humans, including tactile stimulation (Uhl, et al., 1991; Sadato, et al., 2002), olfactory processing (Kupers, et al., 2011), auditory stimulation (Kujala, et al., 1995; Collignon, et al., 2013), speech processing (Röder, et al., 2002), verbal memory (Amedi, et al., 2003), and Braille reading (Sadato, et al., 1996).

In addition to these behavioral adaptations and functional plasticity, structural brain plasticity has been reported in early blind humans. For example, atrophy of the optic tracts, optic nerve, and optic chiasm was observed subjectively in congenitally blind subjects using magnetic resonance imaging (MRI) (Breitenseher, et al., 1998). Using Voxel-Based Morphometry (VBM), a reduction of extrastriate visual areas, optic chiasm, and optic tracts volume was observed in the early blind (Noppeney, et al., 2005; Pan, et al., 2007). Another VBM study showed similar decreases, as well as atrophy of the dorsal lateral geniculate nucleus and pulvinar (Ptito, et al., 2008), two thalamic nuclei involved in visual processing. Volume reduction in the primary and secondary visual areas, motor areas, and anterior/posterior cingulate cortex has also

been reported in the early blind, as measured with deformation-based morphometry (DBM) (Leporé, et al., 2010; Yang, et al., 2014). Together, these results indicate that several visual and non-visual structures are affected by visual deprivation in early blind humans. However, histological differences that underlie macroscopic brain plasticity detected with MRI methods remain unclear.

Using histological analyses, studies conducted in visually deprived rodents evidenced an atrophy of the visual cortex (Rhoades et al., 1984), dorsal lateral geniculate nucleus (Asanuma & Stanfield, 1990) and the superior colliculus (Lund & Lund, 1971; Rhoades, 1980; Smith & Bedi, 1997) as well as a larger volume of the primary auditory cortex (Gyllensten et al., 1966). However, most of animal studies investigating brain plasticity induced by early blindness were conducted either in enucleated (Rhoades et al., 1984; Bronchti et al., 1992; Rauschecker et al., 1992) or dark-reared mice (Gyllensten et al., 1966). One limitation of these models is that cortical subplate neurons are exposed to spontaneous retinal activity (Galli & Maffei, 1988), which may influence cortical development. To avoid this limitation, brain plasticity has been investigated in the

ZRDCT mouse, an anophthalmic mouse strain (Rhoades et al., 1984; Charbonneau et al., 2012). In these studies, however, anophthalmic mice were compared with sighted animals from other strains, which potentially confounds differences induced by blindness with strain differences. To counter this issue, we developed a mouse strain, the ZRDBA mouse, in which half of the offsprings is heterozygous and sighted, while the other half is homozygous and anophthalmic (Touj et al., 2019).

The first aim of the present study was to investigate structural brain plasticity in the whole brain of the ZRDBA mouse using MRI. The second aim of this study was to determine histological characteristics that underlie volume differences detected with MRI between anophthalmic and sighted mice. Based on the above-mentioned studies, we hypothesized that anophthalmic mice would show smaller volumes for the primary and secondary visual cortex and superior colliculi. We also expected larger volumes in nonvisual brain areas including the primary auditory cortex and the amygdala.

Materials and methods

Animals

A total of 42 animals (age: 10-12 weeks), including 22 anophthalmic and 20 normally sighted ZRDBA mice was used for MRI analyses. The anophthalmic group comprised 8 males and 14 females while the sighted group comprised 11 males and 9 females. Among the 42 mice used for MRI, 8 mice per group were used for histological analyses, including 4 females and 4 males in each group. Animals were housed in local facilities with a light/dark cycle of 14 h/10 h. All experimental procedures were approved by the animal care committee of " Université du Québec à Trois-Rivières", in accordance with the guidelines of the Canadian Council on Animal Care.

ZRDBA mouse strain

The ZRDBA mouse strain is obtained by crossbreeding a sighted DBA-6 mouse and a ZRDCT anophthalmic mouse (Touj, et al., 2019). The ZRDCT mouse strain was first described by Chase (Chase and Chase, 1941; Chase, 1942; Chase, 1944). These mice have orbits but neither eyes nor optic nerves. Other brain defects are described in detail in a previous report (Touj, et al., 2019). To obtain a stable ZRDBA strain, several steps were performed. First, a ZRDCT mouse was crossed with a DBA-6 mouse. Then, four other crosses were done: F1 x ZRDCT, F2 x ZRDCT, F3 x ZRDCT, and F4 x ZRDCT. This lead to F5 mice, which were genetically tested for stability and used to maintain the ZRDBA colony. In the ZRDBA colony, about half of the offsprings are born anophthalmic and the other half are born normally sighted when anophthalmic mice are bred with sighted mice. Thus, the ZRDBA strain allows the comparison of anophthalmic and sighted offsprings with the same genetic background.

Structural brain imaging

Mice were deeply anesthetized (4% isoflurane) and perfused through the heart with a 30 ml solution (120 µL of contrast agent (Magnevist®: gadopentetate dimeglumine, Bayer Healthcare, Whippany, NJ, USA) in 0.1 M Phosphate Buffered Saline (PBS)), at a flow rate of 1.0 ml/min. The animal was then perfused with a second 30 ml solution (120 µL of gadopentetate dimeglumine in 4% paraformaldehyde solution (Paraformaldehyde, powder,

95%, Sigma Aldrich, Missouri, USA)), at a flow rate of 1.0 ml/min. Immediately after perfusion, the head was collected and placed in a 30ml solution (120 µL of gadopentetate dimeglumine in 4% paraformaldehyde) at 4°C overnight. Twenty-four hours after perfusion, imaging was conducted at the cerebral imaging center of the Douglas Mental Health University Institute with a Bruker 7T 70/30 USR scanner, with a 30 cm inner bore magnet. The brain was imaged within the skull to minimize geometric distortion. A 32 minute 3D FLASH (Fast, Low Angle SHot) sequence was used with a repetition time (TR) of 20 ms, an echo time (TE) of 4.5 ms, a flip angle of 20°, and a matrix size of 258 by 228 by 130 at 70um isotropic resolution.

Magnetic Resonance Imaging analyses

Images were converted to the MINC file format (http://www.bic.mni.mcgill.ca/ServicesSoftware/MINC), denoised using adaptive nonlocal means (Manjon et al., 2010) and inhomogeneity-corrected using the N4 algorithm (Tustison et al., 2010). Two analysis methods were used to measure structural differences between anophthalmic and sighted mice. Differences in the ROIs, namely the primaryvisual cortex (V1), medial and lateral areas of the secondary visual cortex (V2M and V2L), primary auditory cortex (Au1), left and right superior colliculus (sc), and amygdala (AMY), were examined with the Multiple Automatically Generated Templates (MAGeT) method (Chakravarty et al., 2013). Voxel-wise deformation-based morphometry (DBM) was used to detect more localized changes within brain structures that may be missed by the MAGeT method.

Volumetric analysis with the MAGeT method

Segmentation was performed using the MAGeT method, a modified multi-atlas technique based on the Dorr-Steadman-Ullman atlas (Dorr et al., 2008). Jacobian determinants were log transformed and blurred at 0.2 mm full-width half-maximum in order to conform better to Gaussian assumptions for statistical testing. The MAGeT segmentation algorithm allows accurate segmentation and labeling of the different brain structures and thus, allows the comparison of entire brain structure volumes between groups. The MAGeT method was used to compare volumes of the ROIs between blind and sighted mice.

Voxel-wise deformation-based morphometry

In contrast to the MAGeT method, which compares macroscopic brain structures between groups, deformation-based morphometry (DBM) is a voxel-based analysis. DBM allows detecting localized changes at a mesoscopic scale (several millimeters) with no prior structural segmentation (Ashburner, et al., 1998). Voxel-wise DBM was carried out to warp all individual images onto an average brain to examine group differences (Lerch et al., 2011). Mice brains were registered through a series of nonlinear registrations to create a group-wise average and the Jacobian determinants were estimated to measure local expansions or contractions depending on the deformation magnitude at each voxel.

Histological measurements

Brains were extracted from the skulls and post-fixed in a 4% paraformaldehyde solution for 24 hours and cryoprotected in a 30 % sucrose solution. Coronal sections of 50 µm thickness were collected using a freezing microtome (Leica VT1000S, Leica microsystems, Wetzlar, Germany).

Volumes of the primary visual cortex (V1), medial and lateral areas of the secondary visual cortex (V2M and V2L), primary auditory cortex (Au1), left and right superior colliculus (sc), and amygdala (AMY) were measured on Nissl-stained brain sections under an Olympus BX50W1 microscope coupled to a CCD camera (Optronix, MicroBrightField, Williston, VT, U.S.A.). Delineation of the different structures was performed with the Neurolucida Software version 2019 (MicroBrightField, Williston, VT, U.S.A.). Delineations were based on stereotaxic coordinates (Paxinos and Franklin, 1998) and cytoarchitectural descriptions (Caviness, 1975).

Slices of 50 µm thickness were collected from each brain between Bregma -2.30 and Bregma -4.48 for analysis of V1, V2M, V2L, Au1, and sc. In addition, slices were collected for each brain between Bregma −0.58 and Bregma −2.46 for analysis of the AMY. The contour of each structure was delineated on every other slice (every 100 µm) for a total of 20 slices. To calculate the volume, the surface of each structure of interest on these 20 slices was multiplied by 100 µm to account for the inter-slice gap. These volumes were then summed to obtain the total volume of each structure of interest.

Statistical analyses

Whole brain MAGeT and DBM analyses were performed using the R software package (R Core Team, 2016). Image analysis was performed with the RMINC package Lerch J, 2016). A linear mixed-effect model was used for the voxel-wise analysis of the log-transformed absolute Jacobians to examine volumetric differences between groups with sex as a covariable. The False Discovery Rate (FDR) was used to correct for multiple comparisons across voxels (Benjamini & Hochberg, 1995). The DBM analysis results are illustrated by the q-value, which indicates the highest FDR threshold for a given voxel. Covariance between the volume of visual structures (V1, V2 and superior colliculus) and absolute jacobians across the brain was examined to compare blind and sighted mice using an ANCOVA analysis. The same analysis was performed for a control region, the restrosplenial cortex, an area that is not involved in visual function. Statistical analyses for regions of interest on MRI images and histological slices were performed with Statistica v13.0 (Dell Inc., Tulsa, OK, USA) using mixed ANOVAs with group and sex as between-subject factors. The Fisher post hoc test was used to decompose significant effects. All data are expressed as mean ± SD unless otherwise specified. The statistical threshold was set at $p \leq 0.05$ for significance. Effect size is reported as partial eta squared ($\eta^2 p$).

Results

MRI volume differences for regions of interest

ROI volumes were compared between groups and sexes using a mixed ANOVA. Volumes were different between groups across regions (interaction: $F11.418=7.5$, $p<0.001$, $\eta 2p=0.16$) and this effect was not affected by sex (interaction: $F11.418=1.0$, $p=0.48$, $\eta 2p=0.02$). The Fisher post hoc test revealed significantly smaller volumes in anophthalmic compared with sighted mice for the left but not right V1 ($p=0.03$ and $p=0.10$, respectively) and for the left and right sc (both $p<0.001$) (see Table 1 and Fig. 1). Volumes were not significantly different between groups for other regions of interest (all $p>0.07$; see Table 1).

Table 4-1 Volumetric differences in regions of interest (MRI MAGeT analysis)

Brain area	Mean ± SD blind	Mean ± SD sighted	P-value
Right V1	1.21 ± 0.10	1.30 ± 0.11	0.10
Left V1	1.25 ± 0.10	1.38 ± 0.11	0.03
Right V2M	1,43 ± 0.10	1,51 ± 0.12	0.22
Left V2M	1,15± 0.11	1,20 ± 0.10	0.43
Right V2L	0.60 ± 0.05	0,61 ± 0.04	0.76
Left V2L	0,78 ± 0.05	0,81 ± 0.04	0.60
Right sc	3.67 ± 0.32	4.07 ± 0.27	<0.001
Left sc	3.67 ± 0.28	4.07 ± 0.30	<0.001
Right Au1	0,56 ± 0.06	0,60 ± 0.05	0.57
Left Au1	0,54 ± 0.06	0,53 ± 0.04	0.94
Right AMY	4.54 ± 0.32	4.43 ± 0.30	0.07
Left AMY	5.24 ± 0.28	5.29 ± 0.30	0.31

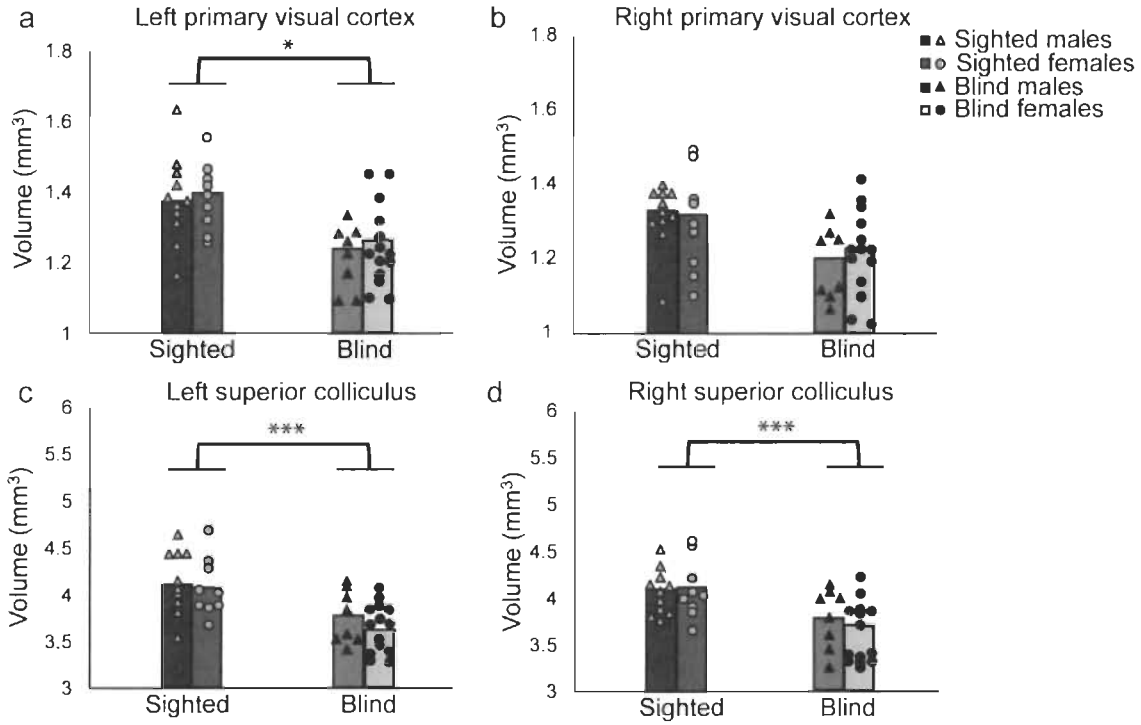

Figure 4-1 MAGeT volumetric differences between groups in the regions of interest

a *The volume of the left V1 was smaller in anophthalmic compared with sighted mice (p= 0.03).*

b *The volume of the right V1 was not significantly different between anophthalmic and sighted*

mice (p=0.10). ***c*** *The volume of the left sc was smaller in anophthalmic compared with sighted mice (p<0.001).* ***d*** *The volume of the right sc was also smaller in anophthalmic compared with sighted mice (p<0.001). There was no significant difference between sexes for either of the structures. *p<0.05 ***p<0.001.* ***sc*** *superior colliculus;* ***V1*** *primary visual cortex.*

Voxel-wise deformation-based morphometry (DBM)

Further MRI volume differences were detected using whole brain DBM analysis. Larger voxel volumes were observed in anophthalmic compared with sighted mice in several brain regions, including the central nucleus of the amygdala (CeA) (bilaterally), cingulate and insular areas, as well as olfactory and orbital areas (see Table 2 and Fig. 2 for details). Smaller volumes in anophthalmic compared with sighted mice were observed in several brain regions including V1, V2L, the dorsal lateral geniculate nucleus (DLG), and cingulate and motor areas (see Table 3 and Fig. 3). No significant group difference was observed between sexes except for in the anterior hypothalamic area (AHA), where the volume was larger in anophthalmic females compared with anophthalmic males.

Table 4-2 Voxel-wise DBM analysis revealing areas larger in blind

Areas larger in blind mice	Hemisphere	t-statistics	q-value
Olfactory and orbital regions			
Granule cell layer of olfactory bulb (Gl)	Bilateral	4.97	<0.01
Glomerular cell layer of olfactory bulb (GrO)	Bilateral	4.76	<0.01
Anterior olfactory area, external part (AOE)	Right	4.27	<0.01
Glomerular layer of accessory olfactory bulb (GlA)	Bilateral	5.97	<0.01
Anterior olfactory area, medial part (AOM)	Right	5.67	<0.01
Mitral cell layer of olfactory bulb (MiA)	Bilateral	7.77	<0.01
Accessory olfactory bulb (AOB)	Right	10.27	<0.01
Ventral orbital cortex (VO)	Bilateral	9.37	<0.01
Lateral orbital cortex (LO)	Right	5.37	<0.01
Medial orbital cortex (MO)	Bilateral	5.17	<0.01
Anterior olfactory area, dorsal part (AOD)	Right	4.07	<0.01
External plexiform layer of accessory olfactory bulb (EPlA)	Bilateral	5.97	<0.01
Olfactory tubercle (TU)	Right	5.57	<0.01
Piriform cortex (Pir)	Bilateral	5.27	<0.01
Cingulate, insular, thalamic and hippocampal regions			
Anterior cingulate cortex , area 2 (Cg2)	Bilateral	5.17	<0.01

Claustrum (Cl)	Left	6.27	<0.01
Dysgranular insular cortex (DI)	Left	5.77	<0.01
Agranular insular cortex, ventral part (AIV)	Left	5.15	<0.01
Ventral medial thalamic nucleus (VM)			
CA 1 of hippocampus (CA1)			
Medial Mammillary nucleus, medial part (MM)	Bilateral	6.37	<0.01
Medial Mammillary nucleus, lateral part (ML)	Bilateral	6.40	<0.01
Fornix (f)	Left	5.17	<0.01
Fimbria (fi)	Bilateral	4.07	<0.01
Dentate gyrus (DG)	Bilateral	5.67	<0.01
Zona Incerta (ZI)	Left	3.37	<0.01
Subcortical regions			
Accumbens Nucleus, core (AcbC)	Left	5.17	<0.01
Accumbens Nucleus, shell (AcbSh)	Right	4.07	<0.01
Caudate putamen (CPu)	Bilateral	5.61	<0.01
Globus Pallidus (GP)	Bilateral	5.57	<0.01
Central amygdaloid nucleus (CeA)	Bilateral	6.77	<0.01
Bed nucleus of stria terminalis (STIA)	Bilateral	7.17	<0.01
Brain stem and cerebellum regions			
Interposed cerebellar nucleus (IntP)	Bilateral	4.37	<0.01
Medial cerebellar peduncle (mcp)	Bilateral	3.37	<0.01
Pontine reticular nucleus, oral (PnO)	Bilateral	6.57	<0.01
Principal sensory trigeminal nucleus (Pr5)	Right	6.27	<0.01
Medial parabrachial nucleus (MPB)	Bilateral	4.27	<0.01
Motor trigeminal nucleus (5N)	Left	4.27	<0.01
Medial cerebellar nucleus (Med)	Bilateral	4.57	<0.01
Lateral parabrachial nucleus (LPB)	Bilateral	4.57	<0.01
Raphe magnus nucleus (RMg)	Medial	3.37	<0.05
Superior cerebellar nucleus (scp)	Right	4.27	<0.01
Spinal trigeminal nucleus, interpolar part (Sp5I)	Bilateral	4.97	<0.01
Gigantocelluar reticular nucleus (Gi)	Bilateral	4.17	<0.01
Grey matter of the paramedian lobule (PM)	Left	3.87	<0.01
Cerebellar white matter (cbw)	Bilateral	5.37	<0.01
White matter of crus cerebellum 2 (crus 2)	Bilateral	4.97	<0.01
White matter of inferior cerebellar peduncle (icp)	Bilateral	5.77	<0.01

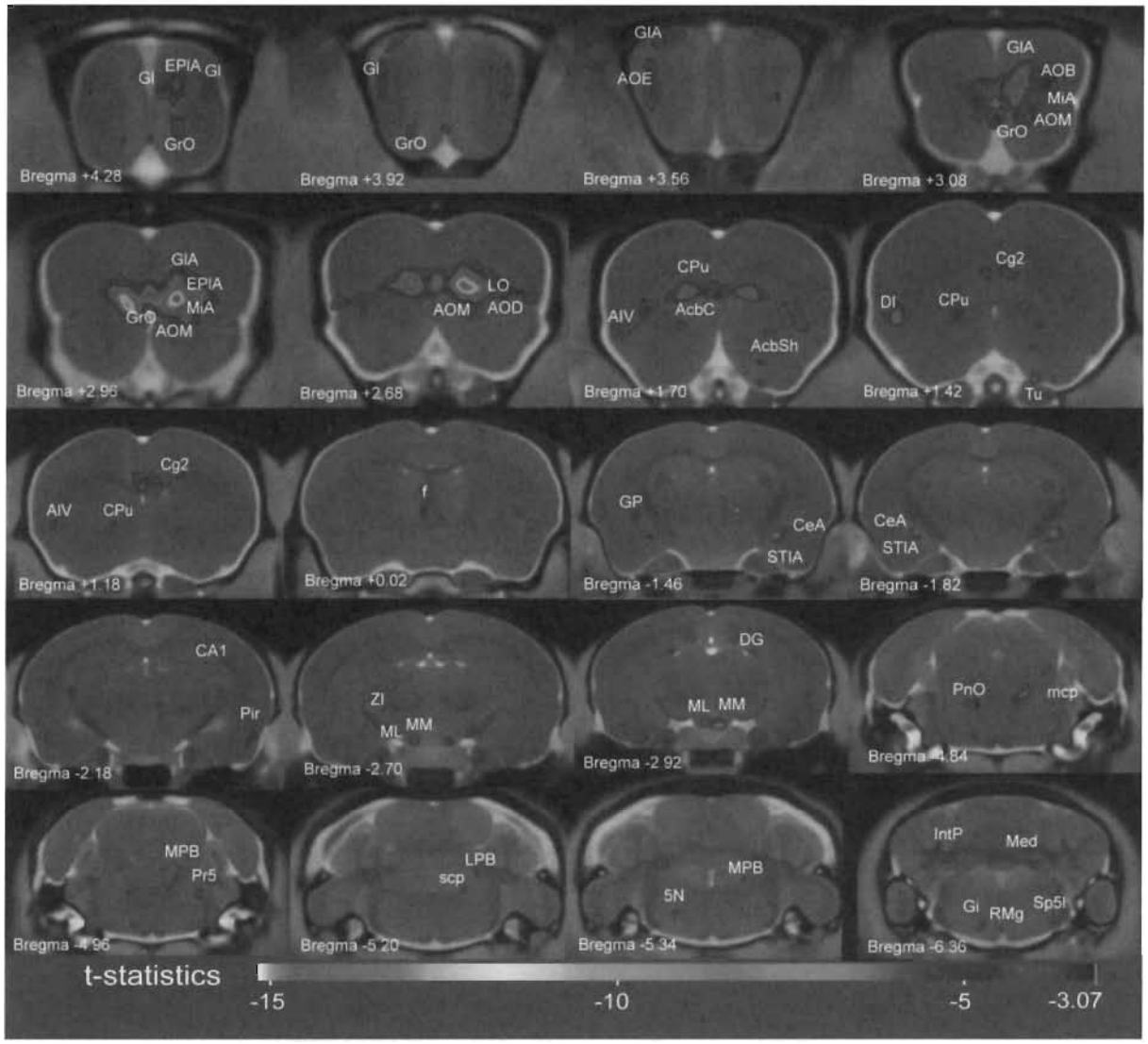

Figure 4-2 DBM analysis revealing structures that were larger in anophthalmic mice

DBM analysis revealed several structures to be larger in anophthalmic compared with sighted mice. Larger volumes were observed in the olfactory and orbital areas as well as in the amygdala and cerebellum. Threshold was set at $p<0.05$ and corrected for multiple comparisons. See Table 3 for further details.

Table 4-3 Voxel-wise DBM analysis revealing areas smaller in blind

Areas smaller in blind mice	Hemisphere	t-statistics	q-value
Visual structures			
Optic tract (opt)	Bilateral	8,91	<0.01
Optic tract (opt)/Cerebral peduncle (cp)	Bilateral	15,21	<0.01
Optic tract (medial to hippocampus)	Bilateral	13,81	<0.01
Cerebral peduncle (cp)	Bilateral	5,61	<0.01
Optic chiasm (och)	Bilateral	4.8	<0.01
Substantia nigra (SNR)	Bilateral	3,71	<0.05
Brachium of the superior colliculus (bsc)	Bilateral	4,51	<0.01
Superior colliculus (sc)	Bilateral	8.21	<0.01
Supraoptic nucleus (SO)	Bilateral	4,41	<0.01
Commissure of the inferior colliculus (cic)	Bilateral	7,21	<0.01
Brachium of the inferior colliculus (BIC)	Bilateral	5,91	<0.01
Dorsal cortex of inferior colliculus (DCIC)	Bilateral	5.89	<0.01
Primary visual cortex (V1)	Bilateral	6,51	<0.01
Secondary visual cortex, lateral area (V2L)	Bilateral	4.31	<0.01
Dorsal lateral geniculate nucleus (DLG)	Bilateral	8.22	<0.01
External cortex of inferior colliculus (ECIC)	Bilateral	5.31	<0.01
Hypothalamic and hippocampal regions			
Lateral septal nucleus (LSI)	Medial	3,41	<0.05
Septohippocampal nucleus (SHi)	Medial	4,81	<0.01
Caudate putamen (CPu)	Left	4,01	<0.01
Ventrolateral preoptic nucleus (VLPO)	Bilateral	7,21	<0.01
Ventromedial preoptic nucleus (VMPO)	Bilateral	5,11	<0.01
Nucleus of the horizontal limb of the diagonal band (HDB)	Left	4,41	<0.01
Medial preoptic area (MPA)	Bilateral	7.54	<0.01
Magnocellular preoptic nucleus (MCPO)	Left	3.22	<0.05
Cuneiform nucleus (CnF)	Bilateral	3,51	<0.05
Anterior hypothalamic area, anterior part (AHA)	Bilateral	4.55	<0.05
Lateral anterior hypothalamic nucleus (LA)	Bilateral	5.12	<0.05
Nucleus of the vertical limb of the diagonal band (VDB)	Bilateral	7,01	<0.01
Ventrolateral hypothalmic nucleus (VLH)	Bilateral	4.60	<0.01
Vascular organ of the lamina terminalis (VOLT)	Medial	9,71	<0.01
Retrosplenial dysgranular cortex (RSD)	Left	3.20	<0.05
Medial Geniculate Nucleurs (MGD)	Right	7.11	<0.01
Cingulate and motor regions			
Primary/secondary motor cortex (M1, M2)	Bilateral	4,51	<0.01
Posterior cingulate cortex, area 2 (Cg2)	Bilateral	5.71	<0.01

Frontal association cortex (FrA)	Bilateral	5,71	<0.01

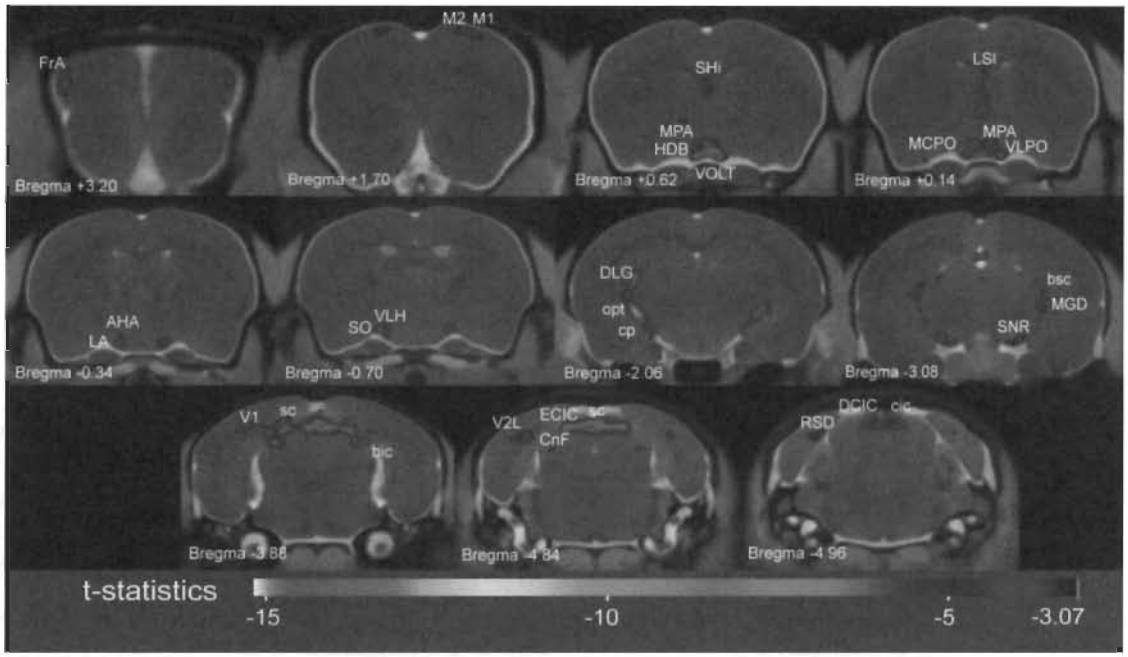

Figure 4-3 DBM analysis revealing structures that were smaller in anophthalmic mice

DBM analysis revealed several structures to be smaller in anophthalmic compared with sighted mice. Smaller volumes were observed in the motor areas, posterior cingulate cortex, optic tracts, primary and secondary visual cortices, and superior colliculus. Threshold was set at $p<0.05$ and corrected for multiple comparisons. See Table 4 for further details.

Covariance analysis

Covariance between visual structures and absolute jacobians across the brain was not significantly different between groups (FDR> 20% for all structures). Similar results were obtained for the control structure (RSC) (FDR>20%).

Histological analyses

From brain slices, the same ROI volumes as used in the MRI analyses (V1, V2M, V2L, sc, Au1 and AMY) were compared between groups and sexes using a mixed ANOVA. ROIs were delineated based on histological features and stereotaxic coordinates (Paxinos and Franklin, 1998). Delineation of the different structures is shown on a representative section in

Fig. 4. Volumes were different between groups across regions (interaction: $F_{11,132}$=10.1, p<0.001, η^2_p=0.46) and this effect was not affected by sex (interaction: $F_{11,132}$=0.5, p=0.93, η^2_p=0.04). The Fisher post hoc test revealed significantly smaller volumes in anophthalmic compared with sighted mice for V1 (see Fig. 5a-b) and V2M (see Fig. 5c-d), bilaterally (p<0.01). Accordingly, atrophy of cortical layer IV was observed in this region (see Fig. 6). Atrophy of the left and right sc was also observed (both p<0.001) (see Fig. 5g-h), and the superficial (visual) layers of the sc were found to be absent in anophthalmic mice (see Fig. 7). Post hoc tests also revealed larger volumes in the left and right amygdala (see Fig. 8c-d) (p<0.01) (see Table 4).Volumes were not significantly different between groups for V2L (see Fig. 5e-f) or Au1 (see Fig. 8a-b) (all p>0.09; see Table 4).

Table 4-4 Volumetric differences in regions of interest (histological analysis)

Brain area	Mean ± SD blind	Mean ± SD sighted	P-value
Right V1	0,84 ± 0.15	1,17 ± 0.09	<0.001
Left V1	0,83 ± 0.15	1,11 ± 0.1	<0.001
Right V2M	1,34 ± 0.12	1,54 ± 0.15	0.01
Left V2M	1,35 ± 0.06	1,55 ± 0.20	0.01
Right V2L	0,74 ± 0.07	0,89 ± 0.06	0.07
Left V2L	0,73 ± 0.10	0,87 ± 0.12	0.10
Right sc	1.54 ± 0.33	1.99 ± 0.18	<0.001
Left sc	1.54 ± 0.29	2.06 ± 0.22	<0.001
Right Au1	0,64 ± 0.07	0,61 ± 0.10	0.67
Left Au1	0,66 ± 0.07	0,62 ± 0.08	0.65
Right AMY	2.12 ± 0.23	1.87 ± 0.13	0.003
Left AMY	2.11 ± 0.27	1.85 ± 0.13	0.001

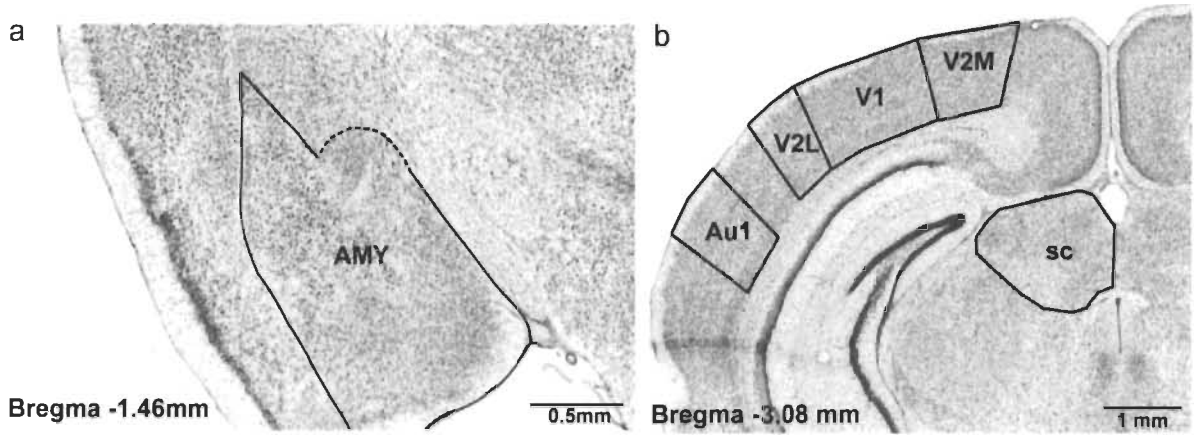

Figure 4-4 Photomicrographs of the delineations of the amygdala, primary and secondary visual cortices, primary auditory cortex, and superior colliculus

a *Representative photomicrographs of the delineation of AMY at Bregma -1.46 mm, based on stereotaxic coordinates of Paxinos and Franklin (1998) (Paxinos and Franklin, 1998).* ***b*** *Representative photomicrographs of the delineations of V1, V2M, V2L, Au1, and sc at Bregma -3.08 mm, based on stereotaxic coordinates of Paxinos and Franklin (1998) (Paxinos and Franklin, 1998).* ***AMY*** *Amygdala;* ***Au1*** *Primary auditory cortex,* ***sc*** *superior colliculus;* ***V1*** *Primary visual cortex;* ***V2L*** *Secondary visual cortex, lateral area;* ***V2M*** *Secondary visual cortex, medial area.*

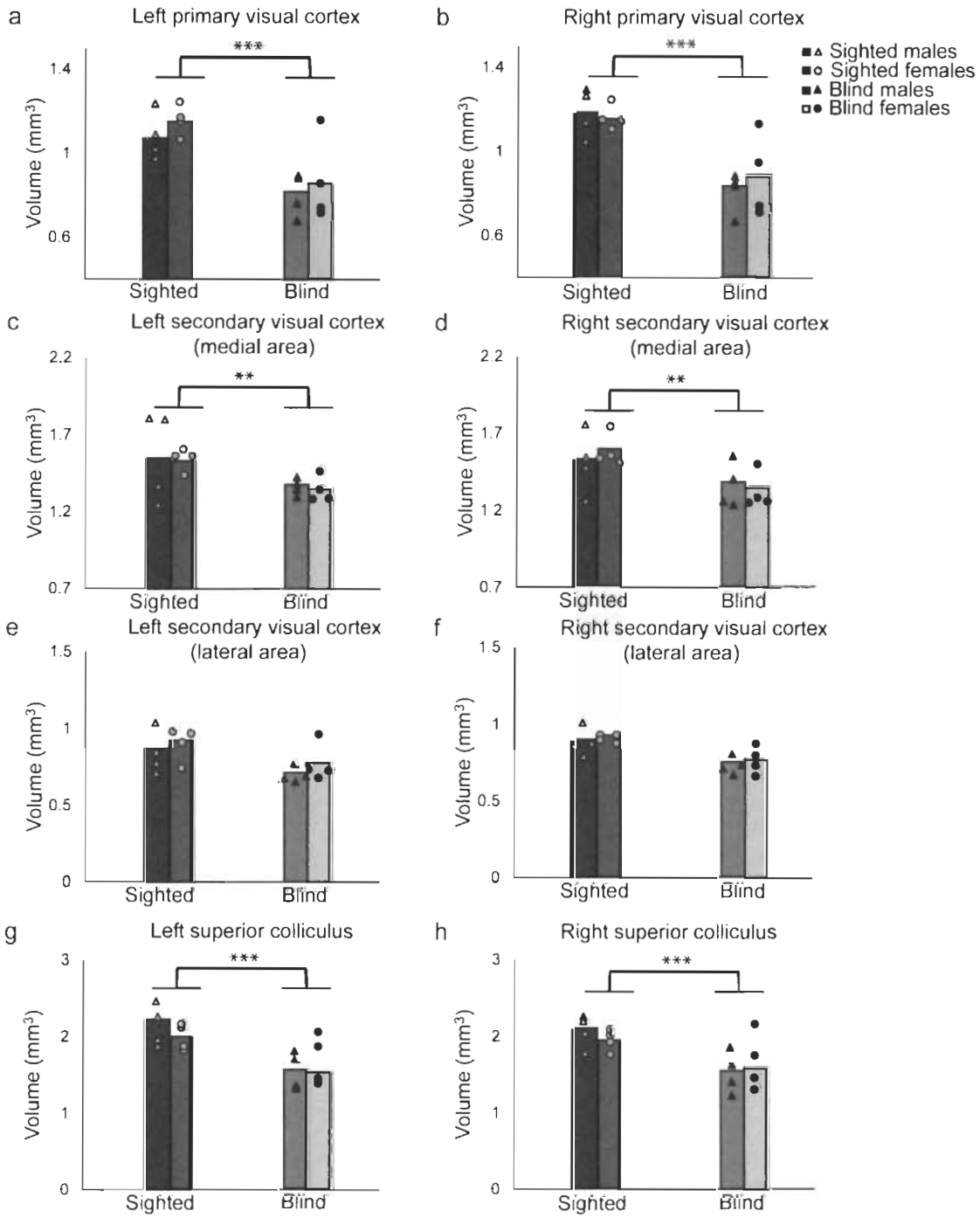

Figure 4-5 Histological analysis showing atrophy of primary and secondary visual cortices and superior colliculus

a *Volumes in left V1 were smaller in anophthalmic compared with sighted mice ($p<0.001$).* ***b*** *Right V1 volumes were smaller in anophthalmic compared with sighted mice ($p<0.001$).* ***c*** *Left V2M volumes were smaller in anophthalmic compared with sighted mice ($p=0.01$).* ***d*** *Right V2M*

volumes were smaller in anophthalmic compared with sighted mice (p=0.01). ***e*** *Left V2L volumes were not significantly different between anophthalmic and sighted mice (p=0.10).* ***f*** *Right V2L volumes were not significantly different between anophthalmic and sighted mice (p=0.07).* ***g*** *Left sc volumes were smaller in anophthalmic compared with sighted mice (p<0.001).* ***h*** *Right sc volumes were smaller in anophthalmic compared with sighted mice (p<0.001). There was no significant difference between sexes for any of the structures. **P<0.01 ***p<0.001.* ***sc*** *superior colliculus;* ***V1*** *Primary visual cortex;* ***V2L*** *Secondary visual cortex, lateral area;* ***V2M*** *Secondary visual cortex, medial area.*

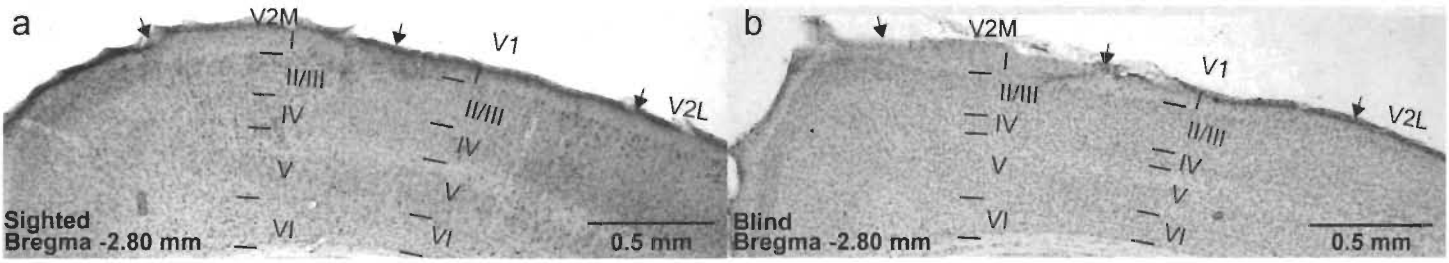

Figure 4-6 Photomicrographs showing atrophy of layer IV in V1 and V2M

a Representative photomicrograph of a sighted mouse at Bregma -2.80 mm showing the different layers (I-VI) of V1 and V2M. **b** Representative photomicrograph of an anophthalmic mouse at Bregma -2.80 mm showing the different layers (I-VI) of V1 and V2M. A narrowing of layer IV is observed in the anophthalmic mouse. **sc** superior colliculus; **V1** Primary visual cortex; **V2M** Secondary visual cortex, medial area.

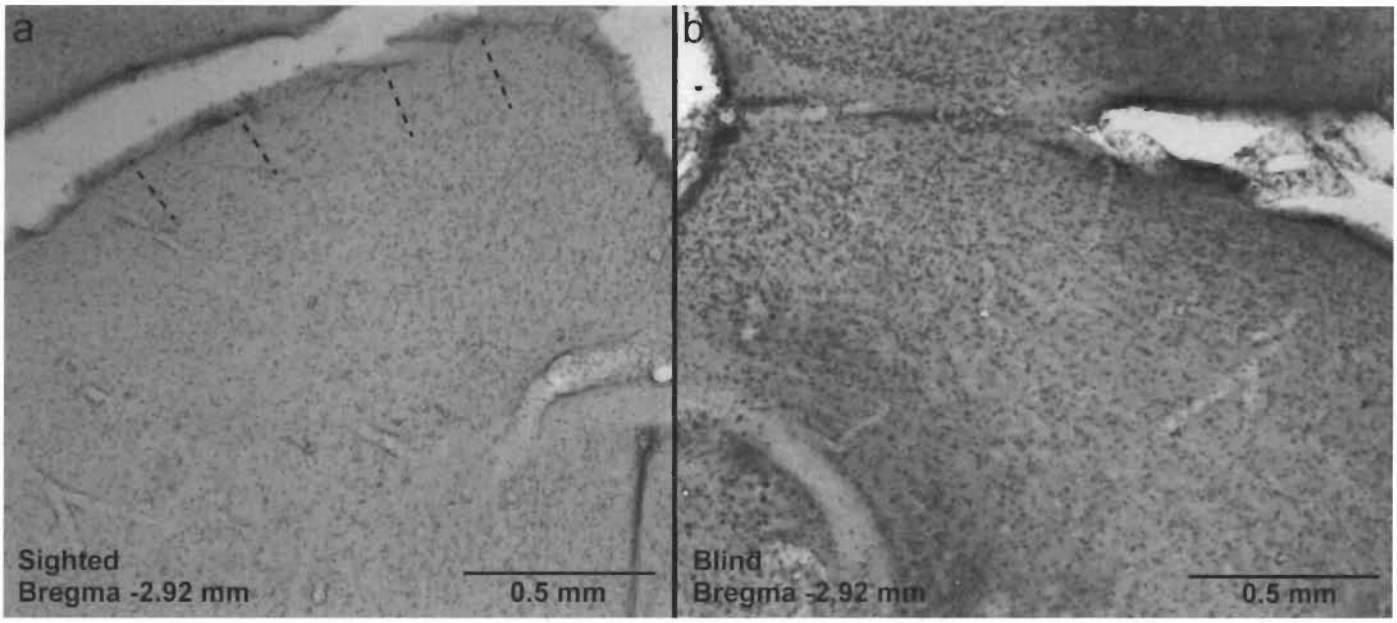

Figure 4-7 Photomicrographs showing atrophy of superficial layers of superior colliculi

a *Representative photomicrograph of a sighted mouse at Bregma -2.92 mm showing the sc. The dotted lines show the superficial layer representing the visual fields in the sc.* ***b*** *Representative photomicrograph of an anophthalmic mouse at Bregma -2.92 mm showing the sc. An absence of the superficial layer representing the visual fields in the sc is observed.* ***sc*** *superior colliculus;* ***V1*** *Primary visual cortex;* ***V2M*** *Secondary visual cortex, medial area.*

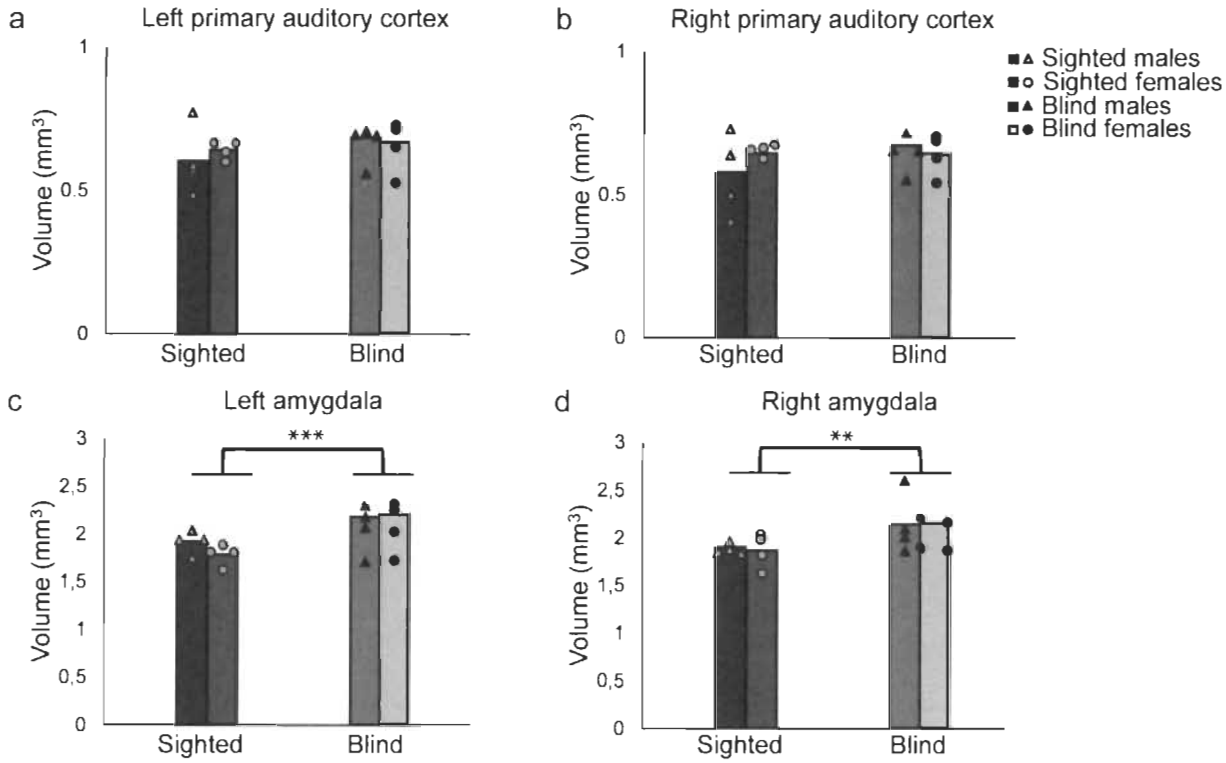

Figure 4-8 Histological analysis showing no difference in the primary auditory cortex and a larger amygdala volume in anophthalmic mice

a *The left Au1 volume was not significantly different between anophthalmic and sighted mice (p=0.67).* ***b*** *The right Au1 volume was not significantly different between anophthalmic and sighted mice (p=0.65).* ***c*** *The left AMY volume was larger in anophthalmic compared with sighted mice (p=0.001).* ***d*** *The right AMY volume was larger in anophthalmic compared with sighted mice (p=0.003). There was no significant difference between sexes for both structures. **P<0.01 ***p<0.001.* ***AMY*** *Amygdala;* ***Au1*** *Primary auditory cortex.*

Discussion

The aim of the present study was to investigate structural brain plasticity in anophthalmic mice issued from a ZRDBA mouse colony. To our knowledge, this is the first study investigating whole brain plasticity in anophthalmic animals. MRI analyses revealed volume differences between anophthalmic and sighted mice, in visual and nonvisual brain regions. Histological analyses of Nissl-stained sections from the same brains revealed specific miscroscopic changes and confirmed MRI findings.

Plasticity in visual structures

In anophthalmic compared with sighted mice, the MAGeT analysis revealed smaller V1 and sc volumes and the DBM analysis revealed smaller volumes in V1, V2L, DLG, and sc. Similar findings were observed using histological analyses for V1, V2M, and sc. These results are consistent with previous histological studies in anophthalmic (Bravo and Inzunza, 1994) and enucleated (Laing, et al., 2012; Andelin, et al., 2018) rats as well as histological (Bock, et al., 2012) and MRI studies in enucleated ferrets (Andelin, et al., 2018) showing a smaller V1 volume in blind compared with sighted animals. Studies in early blind humans have also shown smaller visual structures. For example, V1 was smaller in anophthalmic compared with normally sighted individuals (Andelin, et al., 2018). Moreover, congenitally blind humans showed smaller volumes compared with sighted controls for the optic tracts, DLG, optic chiasm, V1, and V2 (Noppeney, et al., 2005; Pan, et al., 2007; Ptito, et al., 2008; Park, et al., 2009). Other studies evidenced increased cortical thickness in the visual areas (Jiang et al., 2009; Park et al., 2009; Anurova et al., 2015; Qin et al., 2015). The gray matter volume is determined by both cortical thickness and cortical surface area. The development of the occipital cortex is chacterized by an overproduction of synapses which is followed by pruning of inactive synapses during early years of life (Huttenlocher, 1984; Rakic et al., 1986; Bourgeois et al., 1989; Rakic et al., 1994). While synaptogenisis is independent of retinal input, synaptic pruning is driven by visual experience (Huttenlocher, 1984). The increased cortical thickness in the visual areas could be explained by the retention of exuberant connections that is caused by the interruption of synaptic pruning as a result of early visual deprivation (Innocenti, 2007; Park et al., 2009; Anurova et al., 2015). Conversely, the volumetric reduction of the visual areas is more likely the result of deprivation-

related disuse (Noppeney et al., 2005; Voss et al., 2014). This is supported by findings from MRI and histological analyses in the present study.

The present resutls evidenced an atrophy of cortical layer IV in both V1 and V2M in anopthalmic compared with sighted mice, resulting in volumetric differences between groups detected with histology and MRI. Atrophy of layer IV in the visual cortex has been shown in enucleated mice (Valverde, 1968). Layer IV receives thalamic inputs from DLG (Peters & Feldman, 1977) and its atrophy is most likely due to the lack of visual input from DLG, which is also smaller in anophthalmic compared with sighted mice. The absence of visual input to layer IV disrupts the normal development of visual areas, leading to decreased synaptic density, loss of glia (Reillo et al., 2011), loss of dendritic spines, or reduction of the number of axonal arborizations, as described in a study conducted in visually deprived rabbits (Globus & Scheibel, 1966). As visual experience is the principal driver of neuronal maturation in visual areas, the absence of visual input leads to a lack of maturation and reduced expansion (Kolb & Whishaw, 1998). Moreover, the earlier blindness occurs, the smaller the visual areas (Park et al., 2009; Laing et al., 2012; Lazzouni & Lepore, 2014; Andelin et al., 2018). Thus, it could be presumed that the present results are the most extreme case of structural plasticity induced by blindness.

Histological analyses also revealed an absence of the superficial layers of sc, resulting in a smaller volume of sc in anophthalmic compared with sighted mice as easured with histology and MRI. Consistent with this finding, an absence of the superfical layers and a smaller volume of the sc were shown in enucleated hamsters (Rhoades, 1980), enucleated mice (Godement et al., 1980) and blind mole rats. The superficial layers of sc are exclusively visual in most mammals (Drager & Hubel, 1976; Crish et al., 2006) and their atrophy is most likely due to the disruption of retinal input (Crish et al., 2006). In contrast to findings from animal studies, an MRI study showed a preservation of the superior colliculus in congenital blind humans (Cecchetti et al., 2016).

The authors proposed that the lack of volumetric reduction in human sc may be due to reduced retinotectal projections targetting the superficical layers of sc across species (Tiao & Blakemore, 1976; Cecchetti et al., 2016) or by a high proportion of deep layer neurons responding to multisensory inputs (Sparks & Hartwich-Young, 1989). This indicates that the

human sc is more complex compared to other mammals and cannot be regarded as merely a visual structure (Cecchetti et al., 2016).

Volume covariance between visual areas and the rest of the brain

In the present study, volume covariance between visual areas and the rest of the brain was not significantly different between groups. Volume covariance can be an indicator of structural network organization (Voss & Zatorre, 2015). The present results suggest that anatomical networks are preserved between the visual areas and the rest ofthe brain in anophthalmic mice, despite the atrophy observed in some visual areas. A study by Voss et al. (2015) evidenced a reduction of the covariance between the right occipital area and other areas of the dorsal visual stream in early blind humans. This suggests that the mechanisms of structural plasticity may differ between species or that anophthalmy and other types of blindness have different effects on brain structure.

Plasticity in olfactory and auditory areas

DBM analyses revealed larger volumes in several olfaction-related regions including the granule cell and glomerular layers of olfactory bulbs, which are involved in odor detection and odor discrimination (Takahashi, et al., 2018), the anterior olfactory nucleus, which is involved in integration of higher order olfactory information (Lei, et al., 2006), the olfactory tubercle, which is involved in multisensory integration of olfactory and auditory information (Wesson and Wilson, 2010), and the piriform cortex, which is involved in odor processing (Howard, et al., 2009). Only one study has examined plasticity in olfactory structures in the blind. In this study, larger olfactory bulbs were observed in early blind individuals (Rombaux, et al., 2010). In addition, the larger olfactory bulb volume was associated with better olfactory function, suggesting that structural plasticity is linked to behavior (Buschhuter, et al., 2008; Rombaux, et al., 2010; Mazal, et al., 2016). Blind individuals rely more on olfaction and have higher odor awareness compared to sighted individuals (Beaulieu-Lefebvre et al., 2011), as a compensatory mechanism allowing the detection of danger more efficiently. This overuse may lead to a volume increase in the olfactory areas. This is consistent with studies showing that olfactory bulbs' function is highly adaptable and that their volume can change as a function of olfactory

performance and training, as well as after recovering from olfactory dysfunction (Rombaux et al., 2009; Araneda et al., 2016).

No group difference was observed in the primary auditory cortex. This is consistent with previous findings in early blind humans showing no structural difference between blind and sighted individuals in the auditory cortex (Noppeney, et al., 2005; Pan, et al., 2007; Ptito, et al., 2008), although one study did show a decrease in auditory cortex thickness in congenitally blind humans (Park, et al., 2009). While results are somewhat conflicting, the volume of the auditory cortex is generally unaffected by early blindness.

Plasticity in other brain areas

In the present study, a smaller volume was observed for the posterior part of the anterior cingulate cortex, the so-called cingulate eye field, in anophthalmic compared with sighted mice. This area is involved in eye movement control such as triggering intentional saccades (Pierrot-Deseilligny, et al., 2003). The substantia nigra, another area involved in eye movement control (Basso and Sommer, 2011), was also smaller in anophthalmic compared with sighted mice. Thus, smaller volumes in these regions are consistent with the lack of visual input in anophthalmic mice.

An atrophy of the stria terminalis, the preoptic area, and the ventromedial hypothalmus was observed in anophthalmic mice. These areas are involved in the regulation of social behaviors such as aggression, defense, social communication, mating, and parental care (Lebow and Chen, 2016). The stria terminalis is also invoved in regulating fear and anxiety behaviors. To date, there is no data in the ZRDBA mouse on social behaviors and neuronal activity in these regions. Thus, a future avenue of research would be to examine whether structural plasticity in this region impacts function and social behavior in this mouse model.

DBM analyses also revealed an enlargement of the central nucleus of the amygdala in anophthalmic compared with sighted mice; this is an area involved in pain processing (Bernard et al., 1992; Neugebauer & Li, 2002). Furthermore, histological analyses revealed a larger volume of the amygdala in anophthalmic compared with sighted mice. The amygdala is involved in affective and emotional functions as well as in pain processing (Janak & Tye, 2015).

In ZRDBA mice, we previously evidenced hypersensitivity to pain in anophthalmic mice (Touj et al., 2019)., consistent with hypersensitivity to thermal pain in early blind humans (Slimani et al., 2013). In blind ZRDBA mice, increased pain perception was accompanied by increased activity in and larger volume of the amygdala (Touj et al., 2019). In these mice, no significant volume difference was observed for the basolateral and central nuclei of the amygdala between anophthalmic and sighted mice, suggesting that the group difference may rely on cumulative volumetric differences in several amygdaloid subnuclei (Touj et al., 2019). Anatomical changes in the amygdala may contribute to pain hypersensitivity observed in anophthalmic mice. This may reflect a compensatory mechanism allowing the blind to detect and avoid danger in their environment with higher efficiency.

We also observed larger nuclei in mammilary bodies in anophthalmic compared with sighted mice. The mammilary bodies are involved in spatial memory (Rosenstock, et al., 1977; Aggleton, et al., 1995); thus, their enlargement may reflect compensation for visual deprivation, but this remains to be investigated with behavioral tests and measurement of neuronal activity.

Blind humans and animals rely more heavily on remaining non-visual sensory modalities, which might lead to anatomical alterations in several brain areas (Kupers and Ptito, 2014). Accordingly, growing evidence suggests that environmental demands and training lead to changes in grey matter (Draganski, et al., 2004). These alterations include changes in axonal arborization, axon number, myelination, synaptic density, and/or glial cell genesis (Zatorre, et al., 2012). This warrants future study to determine behavioral changes that may be associated with structural brain plasticity observed in anophthalmic mice.

Conclusion

In the present study a mouse model of congenital blindness was used to investigate structural brain plasticity induced by blindness. Structural MRI and histological analyses showed plasticity in several brain areas including vision-related structures (primary visual cortex, optic tracts, DLG, and superior colliculus) and the amygdala, as well as olfactory and orbital areas. This mouse model will allow future investigations of the underlying mechanisms

of intramodal and crossmodal brain plasticity following sensory deprivation, as well as the consequent behavioral changes.

List of abbreviations

5N Motor trigeminal nucleus**; AcbC** Accumbens nucleus, core; **AcbSh** Accumbens nucleus, shell; **AHA** Anterior hypothalamic area, anterior part**; AIV** Agranular insular cortex, ventral part; **AMY** Amygdala; **AOB** Accessory olfactory bulb; **AOD** Anterior olfactory area, dorsal part; **AOE** Anterior olfactory area, external part; **AOM** Anterior olfactory area, medial part; **Au1** Primary auditory cortex; **bic** Brachium of the inferior colliculus**; bsc** Brachium of the superior colliculus**; CA1** Field CA1 of hippocampus; **cbw** Cerebellar white matter; **CeA** Central amygdaloid nucleus**; Cg2** Anterior cingulate cortex , area 2; **cic** Commissure of the inferior colliculus**; Cl** Claustrum**; CnF** Cuneiform nucleus; **cp** cerebral peduncle; **CPu** Caudate putamen**; crus 2** White matter of crus cerebellum 2; **DBM** Deformation Based Morphometry; **DCIC** Dorsal cortex of inferior colliculus**; DG** Dentate gyrus; **DI** Dysgranular insular cortex; **DLG** Dorsal lateral geniculate nucleus; **ECIC** External cortex of inferior colliculus; **EPlA** External plexiform layer of accessory olfactory bulb; **f** Fornix; **fi** Fimbria; **FrA** Frontal association cortex; **Gi** Gigantocelluar reticular nucleus; **Gl** Granule cell layer of olfactory bulb; **GlA** Glomerular layer of accessory olfactory bulb; **GP** Globus pallidus; **GrO** Glomerular cell layer of olfactory bulb; **HDB** Nucleus of the horizontal limb of the diagonal band; **icp** White matter of inferior cerebellar peduncle; **IntP** Interposed cerebellar nucleus; **LA** Lateral anterior hypothalamic nucleus; **LO** Lateral orbital cortex; **LPB** Lateral parabrachial nucleus; **LSI** Lateral septal nucleus**; M1** Primary motor cortex; **M2** Secondary motor cortex; **MAGeT** Multiple Automatically Generated Templates; **mcp** Medial cerebellar peduncle; **MCPO** Magnocellular preoptic nucleus; **Med** Medial cerebellar nucleus; **MiA** Mitral cell layer of olfactory bulb; **MGD** Medial geniculate nucleus; **ML** Medial mammillary nucleus, lateral part; **MM** Medial mammillary nucleus, medial part; **MO** Medial orbital cortex; **MPA** Medial preoptic area; **MPB** Medial parabrachial nucleus; **MRI** Magnetic Resonance Imaging; **och** Optic chiasm; **opt** Optic tract; **PBS** Phosphate Buffer Saline; **Pir** Piriform cortex; **PM** Grey matter of the paramedian lobule; **PnO** Pontine reticular nucleus, oral; **Pr5** Principal sensory trigeminal nucleus; **RMg** Raphe magnus nucleus; **ROI** Region of interest; **RSD** Retrosplenial dysgranular cortex; **rTMS** Repetitive transcranial magnetic stimulation; **sc** Superior colliculus; **scp** Superior cerebellar

nucleus; **SHi** Septohippocampal nucleus; **SNR** Substantia nigra; **SO** Supraoptic nucleus; **Sp5I** Spinal trigeminal nucleus, interpolar part; **STIA** Bed nucleus of stria terminalis; **TU** Olfactory tubercle; **V1** Primary visual cortex; **V2L** Secondary visual cortex, lateral area; **V2M** Secondary visual cortex, medial area**; VBM** Voxel Based Morphometry; **VDB** Nucleus of the vertical limb of the diagonal band; **VLH** Ventrolateral hypothalmic nucleus; **VLPO** Ventrolateral preoptic nucleus; **VM** Ventral medial thalamic nucleus; **VMPO** Ventromedial preoptic nucleus; **VO** Ventral orbital cortex; **VOLT** Vascular organ of the lamina terminalis**; ZI** Zona incerta.

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Chapitre V Discussion

Les travaux de cette thèse ont permis de mettre en évidence une modification comportementale olfactive et nociceptive chez les souris aveugles ainsi qu'une plasticité cérébrale dans les structures visuelles et non visuelles. Ces découvertes viennent enrichir les données disponibles sur les changements comportementaux et cérébraux chez les aveugles précoces.

V.1 Amélioration de la fonction olfactive et plasticité cérébrale olfactive chez les souris aveugles

V.1.1 Meilleures performances olfactives chez les souris aveugles

Les caractéristiques de la perception olfactive sont certainement différentes entre l'homme et la souris, ce qui représente une limite dans les études comparant les deux espèces. Cependant, chez tous les mammifères, l'olfaction joue un rôle clé dans le comportement social, l'apport alimentaire et la fuite des prédateurs (Hebb, et al., 2004; Vosshall, 2005; Small, et al., 2008). Dans le cas de la cécité, l'olfaction est particulièrement importante pour ces derniers aspects. En outre, des études ont montré plusieurs similitudes entre l'olfaction chez l'homme et la souris. En fait, les humains et les souris partagent plus de 60% des gènes des récepteurs olfactifs (Godfrey, et al., 2004) et ils ont des préférences olfactives similaires (Mandairon, et al., 2009). Ainsi, l'utilisation d'un modèle de souris de cécité précoce permet d'examiner davantage la plasticité olfactive comportementale et cérébrale dans le cas de privation visuelle.

Dans la première étude de cette thèse, les performances olfactives chez les souris aveugles ont été évaluées à l'aide de trois tests: le test de recherche de nourriture, le test de seuil olfactif ainsi que le test de performance olfactive. Les résultats des analyses ont indiqué une meilleure performance olfactive des souris aveugles comparées aux souris voyantes dans le test de recherche de nourriture ainsi que le test de performance olfactive. Il n'y avait pas de différence entre les groupes dans le test de seuil olfactif.

Nos résultats sont en accord avec les études réalisées chez l'humain qui n'ont pas rapporté de différences entre les aveugles et les voyants en ce qui concerne le seuil olfactif (Griesbach, 1899;

Cherubino and Salis, 1957; Boccuzzi, 1962; Rosenbluth, et al., 2000; Wakefield, et al., 2004; Luers, et al., 2014). Seulement trois études ont rapporté un seuil olfactif plus bas chez les sujets aveugles comparés aux voyants (Cuevas, et al., 2010; Beaulieu-Lefebvre, et al., 2011; Comoglu, et al., 2015). L'absence de différence dans le seuil olfactif entre les personnes aveugles et les voyantes pourrait s'expliquer par la différence de traitement olfactif entre la tâche du seuil olfactif et les tâches de détection et discrimination des odeurs. Ces dernières nécessitent un traitement olfactif de haut de niveau tandis que le seuil olfactif n'implique pas de fonctions cognitives complexes (Hedner, et al., 2010; Sorokowska, et al., 2018). La tâche du seuil olfactif semble plutôt dépendre de mécanismes olfactifs périphériques (Whitcroft, et al., 2017; Sorokowska, et al., 2018).

Les humains et animaux aveugles accordent, en général, plus d'attention que les voyants aux signaux non-visuels externes, y compris les signaux olfactifs (Cuevas, et al., 2009; Beaulieu-Lefebvre, et al., 2011; Kupers, et al., 2011; Gagnon, et al., 2015b). Cela conduit à un traitement amélioré et plus efficace des informations olfactives environnantes. Dans notre étude, dans le test de performance olfactive, le temps d'exploration plus élevé sur les odeurs à valence positive chez les souris aveugles pourrait refléter des processus attentionnels accrus. Les souris aveugles auraient tendance à explorer et analyser davantage les odeurs nouvellement introduites dans leur environnement comparées aux souris voyantes. Le temps d'exploration sur l'odeur aversive 2MB était moins élevé chez les souris aveugles comparées aux voyantes indiquant leur capacité améliorée à identifier les odeurs à valence négative. Cela indiquerait également, leur aptitude améliorée à éviter le danger dans leur environnement ce qui représente une compétence de survie compensatoire. Dans l'ensemble, les résultats du test de recherche de nourriture et du test de performance olfactive indiquent une meilleure conscience et discrimination des odeurs chez les souris aveugles.

Nos résultats s'accordent avec les études montrant une meilleure identification, discrimination et conscience des odeurs chez l'humain aveugle (Murphy and Cain, 1986; Rosenbluth, et al., 2000; Schwenn, et al., 2002; Wakefield, et al., 2004; Rombaux, et al., 2010; Gagnon, et al., 2015a). Chez l'animal, une étude réalisée par Zhou et al. a montré que la privation visuelle postnatale chez les souris résulte en une diminution de la latence de recherche de nourriture

(même test utilisé dans notre étude) et une meilleure discrimination olfactive (Zhou, et al., 2017).

Contrairement à ces études, d'autres recherches investiguant l'identification et discrimination des odeurs chez l'humain, n'ont pas réussi à montrer des différences entre les aveugles et les voyants (Sorokowska, et al., 2018). En fait, dans les études réalisées chez l'humain, les résultats semblent dépendre du paradigme expérimental employé. S'il s'agit d'une identification avec des choix préétablis, la cécité ne semble pas affecter les performances (Smith, et al., 1993; Schwenn, et al., 2002; Beaulieu-Lefebvre, et al., 2011; Comoglu, et al., 2015; Sorokowska, 2016). Cependant, quand il s'agit d'une identification libre des odeurs (liste de choix des odeurs), les aveugles montrent des meilleures performances comparés aux voyants (Murphy and Cain, 1986; Rosenbluth, et al., 2000; Wakefield, et al., 2004; Rombaux, et al., 2010; Renier, et al., 2013; Gagnon, et al., 2015a). Certains chercheurs ont suggéré que ces résultats reflètent plutôt un accès plus rapide à l'information sémantique (Cuevas, et al., 2009). En outre, une méta-analyse de Sorokowska et al. (2018) a montré qu'il n'y a pas de différence dans la perception olfactive entre les aveugles et les voyants. Les auteurs ont montré que la plupart des études incluses dans la méta-analyse qui montrent des meilleures performances olfactives chez les aveugles présentent des biais. Cependant, jusqu'à présent, le nombre d'études examinant les différences olfactives entre les aveugles et les voyants restent peu nombreuses en plus d'être divergentes et hétérogènes ce qui ne permet pas de tirer une conclusion claire (Sorokowska, et al., 2018). La divergence des résultats est due en partie à la non-standardisation des procédures des tests (Beaulieu-Lefebvre, et al., 2011). En fait, une variété de tests a été utilisée pour tester l'olfaction chez l'humain allant des dilutions artisanales aux tests validés comme le test d'odorat de l'université de Pennsylvanie (UPSIT) (Doty, et al., 1981) et le test de Sniffin' Sticks (bâtons d'odeurs) (Hummel, et al., 1997). Une grande partie des études citées plus haut a utilisé le test Sniffin' Stick ou le UPSIT afin d'évaluer les performances olfactives chez les aveugles. Ces tests sont plutôt conçus pour évaluer le dysfonctionnement olfactif chez les patients souffrant de troubles olfactifs. En l'absence de troubles olfactifs, ces tests sont faciles à compléter résultant ainsi en un effet plafond qui atténue toute différence (si existante) entre les groupes (aveugles et voyants) (Manescu, et al., 2018). Ces tests ne sont donc pas l'outil adéquat pour tester les performances olfactives supranormales chez les aveugles. Ça serait probablement plus

pertinent de comparer les aveugles aux voyants en dehors du contexte du laboratoire, dans un environnement plus général et avec des tâches plus écologiques (Sorokowska, et al., 2018). Plusieurs chercheurs ont également suggéré que les résultats incohérents dans les études pourraient être liés à la petite taille des échantillons (nombre de participants) (Luers, et al., 2014; Sorokowska, 2016; Sorokowska, et al., 2018). D'autres facteurs peuvent également influencer les résultats des études comme l'âge de la cécité, le degré de déficience visuelle ainsi que les différences interindividuelles qui présentent un biais important. Il a déjà été montré que les conditions environnantes ainsi que les habitudes individuelles, telles que l'activité physique, peuvent influencer les perceptions sensorielles chez une personne aveugle (Seemungal, et al., 2007). Pour les études futures, il serait important de contrôler ces facteurs pour avoir des échantillons plus homogènes. Les modèles animaux présentent également une autre alternative. Le modèle de souris aveugle précoce ZRDBA, utilisée dans notre étude, permet de contrôler ces facteurs et de tester de façon directe l'impact de la cécité sur la perception olfactive.

La meilleure discrimination et identification des odeurs chez les souris aveugles, observée dans notre étude, est un mécanisme adaptatif de survie qui leur permet de trouver la nourriture et détecter tout danger potentiel dans leur environnement.

Ces performances olfactives améliorées sont étroitement liées à la plasticité cérébrale dans les régions olfactives observée chez les aveugles.

V.1.2 Plasticité cérébrale associée à l'amélioration des fonctions olfactives

Les analyses des résultats de l'histologie ont révélé une augmentation du volume des bulbes olfactifs. De plus, les résultats des analyses IRM ont révélé une augmentation du volume au sein de différentes régions impliquées dans le traitement olfactif, notamment, les bulbes olfactifs, le cortex piriforme et le cortex orbitofrontal.

Les études chez les animaux (Wilson, et al., 2004) et chez les humains (Plailly, et al., 2012) prouvent que le système olfactif est très plastique et peut subir des changements neuronaux et anatomiques suite aux modifications dans l'environnement (Rosselli-Austin and Williams, 1990; Wilson, et al., 2004; Rombaux, et al., 2010; Huart, et al., 2013; Araneda, et al., 2016; Zhou, et al., 2017). Ces modifications anatomiques sont associées aux modifications des performances olfactives. La taille des bulbes olfactifs est, en effet, étroitement reliée à la

fonction olfactive (Huart, et al., 2013). Buschhüter et al. (2008) ont montré que la taille des bulbes olfactifs corréle avec la performance olfactive. Les mêmes auteurs ont également montré que le volume des bulbes olfactifs décroit avec l'âge menant à une diminution des fonctions olfactives (Buschhuter, et al., 2008). Dans une revue récente, les auteurs ont montré que la taille des bulbes olfactifs ne corréle pas spécifiquement avec une composante olfactive (seuil, discrimination, identification) mais plutôt avec la performance olfactive générale (Mazal, et al., 2016). Chez les aveugles précoces, Rombaux et al. (2010) ont montré que l'hypertrophie des bulbes olfactifs corréle avec des meilleures performances olfactives. Zhou et al. (2017) ont montré que chez les rats privés visuellement, l'amélioration des performances olfactives est accompagnée d'une plasticité fonctionnelle dans les bulbes olfactifs ainsi que dans le cortex piriforme (augmentation des potentiels des champs locaux). Une étude par Frasnelli et al. (2010) a montré que la performance olfactive corrèle avec une plasticité structurelle dans d'autres structures cérébrales impliquées dans le traitement chimiosensoriel telles que le cortex orbitofrontal, le cortex entorhinal, le cortex occipital ainsi que l'insula.

En accord avec ces données, dans la présente étude nous avons observé que l'amélioration des performances olfactives chez les souris aveugles est accompagnée d'une augmentation du volume des bulbes olfactifs mise en évidence par les analyses histologiques. Les résultats des analyses IRM de la troisième étude ont également révélé une augmentation du volume des couches cellulaires granulaires et glomérulaires des bulbes olfactifs chez les souris aveugles ce qui pourrait expliquer l'augmentation du volume des bulbes olfactifs mise en évidence en histologie. Les couches cellulaires granulaires et glomérulaires jouent un rôle important dans la fonction olfactive. Une étude récente a montré que ces cellules expriment le gène *oncofetal trophoblast glycoprotein 5T4*, impliqué dans la discrimination olfactive (Takahashi, et al., 2018) permettant de relier ces résultats avec ceux de notre étude comportementale.

Les résultats des analyses IRM ont également montré une augmentation du volume au niveau du noyau olfactif antérieur. Ce dernier, reçoit des inputs directs des cellules mitrales des bulbes olfactifs et intervient dans l'intégration des inputs olfactifs complexes de haut niveau (Lei, et al., 2006). En outre, nous avons observé une augmentation du volume au niveau du tubercule olfactif chez les souris aveugles. Une étude a montré que 65% des unités de cette région répondent aux stimuli olfactifs (Wesson and Wilson, 2010).

Les bulbes olfactifs sont le premier relais de l'information olfactive qui est acheminée, en premier lieu, au cortex piriforme qui l'achemine à son tour à différentes structures cérébrales dont le cortex orbitofrontal, l'amygdale, l'hypothalamus, l'insula, le cortex entorhinal ainsi que l'hippocampe (Scalia and Winans, 1975; Brescia and Seiden, 2009). Dans notre étude, nous avons pu observer une augmentation de volume dans plusieurs de ces régions.

Parmi ces régions le cortex orbitofrontal impliqué dans le traitement des informations visuelles, somatosensorielles, gustatives et olfactives (Rolls, 2004). Plusieurs études ont appuyé le rôle important du cortex orbitofrontal dans l'olfaction. En effet, une lésion dans la région orbitofrontale résulte en une altération de la fonction olfactive (Zatorre and Jones-Gotman, 1991; Li, et al., 2010). En outre, une étude d'IRM fonctionnelle a montré une activation du cortex orbitofrontal, du cortex piriforme, du thalamus et de l'hippocampe durant des tâches de traitement olfactif (Kupers, et al., 2011). En accord avec ces données, nous avons observé une augmentation volumétrique au niveau du cortex piriforme ainsi que le thalamus. Le cortex piriforme est en effet, considéré comme étant le cortex olfactif primaire et son rôle dans l'olfaction a été mis en évidence dans plusieurs études (Haberly, 1998; Sobel, et al., 1998; Stettler and Axel, 2009; Zelano, et al., 2011). De plus, la région médiodorsale du thalamus constitue un relais entre le cortex piriforme et le cortex orbitofrontal et est également impliquée dans la modulation olfactive attentionnelle (Plailly, et al., 2008). Dans la deuxième étude de cette thèse, les analyses histologiques ont montré une augmentation du volume de l'amygdale chez les souris ZRDBA. L'amygdale est impliquée dans le traitement cognitif et émotionnel des odeurs ainsi que dans la mémoire olfactive (Zald and Pardo, 1997; Sevelinges, et al., 2004; De Araujo, et al., 2005). En outre, chez les aveugles précoces une activation plus importante de l'amygdale a été observée pendant une tâche olfactive (Kupers, et al., 2011).

Toutes ces observations mènent à suggérer que l'hypertrophie des bulbes olfactifs et des autres régions impliquées dans le traitement olfactif pourrait expliquer l'amélioration des performances olfactives chez les souris aveugles ZRDBA. .

V.2 Hypersensibilité à la douleur et modifications fonctionnelles et structurelles de l'amygdale

V.2.1 Hypersensibilité à la douleur chez les souris aveugles

Dans la première partie de la deuxième étude de cette thèse, la sensibilité à la douleur mécanique, chimique et thermique chez les souris aveugles a été testée. Les souris aveugles ont montré une hypersensibilité à la douleur dans les trois modalités testées. Ces résultats sont en accord avec une étude antérieure menée chez l'humain (Slimani, et al., 2013). Dans l'étude de Slimani et al., (2013), la sensibilité à la douleur thermique (chaud et froid) a été testée chez des participants aveugles congénitaux et chez des voyants contrôles et les résultats ont mis en évidence une augmentation de la perception de la douleur ainsi qu'une diminution du seuil de douleur chez les aveugles comparés aux voyants. Une étude de la même équipe a montré que cette modulation à la hausse de la sensibilité à la douleur n'était pas présente chez les aveugles tardifs (Slimani, et al., 2014) soulignant l'importance de l'âge de la cécité dans la modification comportementale. L'hypersensibilité à la douleur, comme pour les autres modalités sensorielles supranormales chez les aveugles, est reliée à une plasticité cérébrale.

Dans la deuxième partie de cette étude, nous avons investigué la plasticité structurelle et fonctionnelle de l'amygdale, l'une des structures clés dans le traitement et modulation de la douleur.

V.2.2 Plasticité structurelle et fonctionnelle dans l'amygdale

Chez les souris aveugles ZRDBA, en plus de l'hypersensibilité à la douleur, nous avons observé des changements fonctionnels et structurels dans l'amygdale. Une augmentation de l'activité neuronale induite par la douleur a été mise en évidence par le marquage de la protéine c-Fos au niveau du CeA ainsi que toute l'amygdale. En outre, les analyses histologiques ont révélé une augmentation du volume de l'amygdale chez les souris aveugles. Cette plasticité structurelle pourrait être reliée aux changements comportementaux. Des modifications anatomiques dans différentes régions cérébrales sont observées chez les aveugles précoces (Ptito, et al., 2008; Rombaux, et al., 2010). Cette plasticité anatomique est un mécanisme adaptatif qui implique souvent des changements comportementaux tels que l'augmentation de

la taille des bulbes olfactifs qui corrèle avec les meilleures performances olfactives (Rombaux, et al., 2010) ou l'augmentation de la taille de l'hippocampe qui corrèle avec une meilleure navigation spatiale (Fortin, et al., 2008). Par analogie, l'hypertrophie de l'amygdale observée pourrait contribuer avec la plasticité fonctionnelle à l'hypersensibilité à la douleur mise en évidence chez les souris aveugles.

Dans notre étude, une plus grande activité c-Fos induite par la douleur a été observée chez les souris aveugles, dans le complexe amygdalien et dans CeA. L'implication de l'amygdale et de son noyau central dans le traitement et la modulation de la douleur a été mise en évidence dans plusieurs études. Chez l'humain, les études de neuroimagerie ont démontré une activation de l'amygdale en réponse aux stimuli mécaniques, thermiques et chimiques (capsaicine) (Simons, et al., 2014). Chez les modéles animaux, une étude réalisée chez un modèle de souris d'inflammation chronique a montré que l'activation des ERK au niveau de l'amygdale joue un rôle crucial dans la modulation de l'hypersensibilité périphérique (Carrasquillo and Gereau, 2007). Une autre étude plus récente a montré à l'aide d'approches chimiogénétique la contribution de l'amygdale dans la douleur spontanée chez un modèle de souris de douleur inflammatoire (Arimura, et al., 2019). Plusieurs autres études chez l'animal, ont mis en évidence une augmentation de l'activité fonctionnelle au niveau de l'amygdale en réponse à des stimuli nociceptifs incluant des stimulations mécaniques ou thermiques (Bernard, et al., 1992; Neugebauer and Li, 2002), viscérales (Suwanprathes, et al., 2003; Han and Neugebauer, 2004; Myers and Greenwood-Van Meerveld, 2007; Sadler, et al., 2017), injection de formaline intraplantaire (Carrasquillo and Gereau, 2007; Adedoyin, et al., 2010), douleurs musculaires (Cheng, et al., 2011) et douleur chronique (Goncalves, et al., 2008; Goncalves and Dickenson, 2012).

Des études employant différentes techniques de physiologie et immunohistochimie ont montré que la plasticité liée à la douleur dans CeA et dans l'ensemble de l'amygdale est la conséquence d'une plasticité synaptique (Neugebauer, et al., 2003; Bird, et al., 2005; Han, et al., 2005; Fu and Neugebauer, 2008; Ren, et al., 2013). Ces études ont montré une augmentation de la transmission synaptique excitatrice PB-CeLc (noyau latéral capsulaire du noyau central de l'amygdale), LA-BLA et BLA-CeLc (Bird, et al., 2005; Han, et al., 2005; Fu and Neugebauer, 2008; Ren and Neugebauer, 2010), une diminution de de l'inhibition des neurones du CeLc (Ren

and Neugebauer, 2010; Ren, et al., 2013) ainsi qu'une augmentation de l'excitabilité neuronale dans CeLc (Neugebauer, et al., 2003; Bird, et al., 2005; Fu and Neugebauer, 2008) et BLA (Ji, et al., 2010). Il faut noter que ces changements synaptiques n'ont pas été retrouvés dans tous les modèles de douleur. La neuroplasticité dans l'amygdale diffère selon le type de la douleur (chronique, aiguë, inflammatoire, viscérale...). Dans notre étude, d'autres analyses sont nécessaires fin de déterminer les mécanismes sous-jacents à l'augmentation de l'activité induite par la douleur dans CeA et dans l'ensemble de l'amygdale chez les souris aveugles.

V.3 Plasticité cérébrale chez les souris aveugles dans les structures visuelles et non visuelles

V.3.1 Plasticité cérébrale dans les structures visuelles

Dans la troisième étude, nous avons examiné les changements anatomiques dans les structures visuelles et tout le cerveau à l'aide d'analyses IRM ainsi que des analyses histologiques complémentaires. Les analyses des résultats de l'histologie ont révélé une atrophie dans V1, V2M et les collicules supérieurs. Les analyses des résultats de l'IRM structurelle ont révélé une atrophie dans V1, V2L, DLG, collicules supérieurs, chiasme et tracts optiques. Ces résultats sont en accord avec plusieurs études réalisées chez l'humain et l'animal montrant des réductions de volume similaires. Une réduction volumétrique de différentes structures visuelles incluant DLG, tracts et chiasme optique ainsi que V1 a été mise en évidence chez l'humain aveugle précoce (Noppeney, et al., 2005; Ptito, et al., 2008; Park, et al., 2009; Cecchetti, et al., 2016). Chez les animaux, une atrophie de V1 a été également observée chez le furet énucléé (Bock, et al., 2012; Andelin, et al., 2018), le rat anophtalme (Bravo and Inzunza, 1994) et le rat énucléé (Laing, et al., 2012; Andelin, et al., 2018).

Dans notre étude les analyses histologiques ont montré que l'atrophie de V1 et du V2M est due à une atrophie de la couche corticale IV. Une atrophie de la couche IV a déjà été mise en évidence chez les souris énucléées (Valverde, 1968). En fait, la couche IV reçoit les inputs visuels du DLG (Peters and Feldman, 1977) et dans l'absence d'inputs de DLG, atrophié à son tour, cette couche est atrophiée. Nous avons aussi observé dans la présente étude une atrophie

des couches visuelles superficielles des collicules supérieurs chez les souris aveugles résultant en l'atrophie des collicules supérieurs. Les couches superficielles des collicules supérieurs représentent les champs visuels et leur absence dans le cas de privation visuelle a été mise en évidence chez les hamsters énucléés (Rhoades, 1980) ainsi que chez les souris énucléées (Godement, et al., 1980) .

Cette réduction volumétrique est probablement due à une diminution des cellules gliales (Reillo, et al., 2011), la myélinisation ou/et de l'arborisation axonale ou dendritique comme décrit dans une étude antérieure chez des animaux privés de vision (Globus and Scheibel, 1966). Comme l'expérience visuelle est le principal stimulant de la maturation neuronale dans ces structures, le défaut de la maturation de la matière grise est dû à l'absence d'inputs visuels. L'atrophie des structures visuelles est donc probablement due à l'absence d'input du DLG atrophié qui, à son tour, ne reçoit plus d'input des tracts optiques, également atrophiés menant ainsi à une diminution de l'une ou plusieurs des populations du neuropile. La dépendance de la maturation des structures visuelles de l'expérience visuelle est appuyée par les études qui montrent que le degré d'altération dépend de l'âge de la cécité (Park, et al., 2009; Voss and Zatorre, 2012a; Lazzouni and Lepore, 2014; Li, et al., 2017; Andelin, et al., 2018).

La plasticité dans les structures visuelles est beaucoup plus importante si la cécité survient avant la période critique, qui diffère d'une espèce à l'autre. Une étude récente a comparé la surface du cortex visuel chez différentes espèces (rats, furets et humains) à différents âges de cécité (Andelin, et al., 2018). Les résultats de cette étude montrent une corrélation positive entre l'âge de la cécité et l'expansion de V1 (Andelin, et al., 2018). Au-delà d'une période critique déterminée pour chacune des espèces testées, l'expansion corticale n'est plus affectée par la cécité. Cette période se situe autour du 4^{éme} jour post natal chez les rats, la 3^{éme} semaine postnatale chez les furets (Andelin, et al., 2018) et le premier jour postnatal chez la souris (Chabot, et al., 2007). Chez l'humain, cette période est plus difficile à déterminer. À l'aide d'une méta-analyse incluant différentes études chez différentes espèces, Andelin et al. (2018) suggèrent que cette période se situe autour de la 17^{éme} semaine chez l'humain en ce qui concerne l'expansion corticale. Certains auteurs suggèrent que cette période peut s'étendre jusqu'à l'âge de 5 ans chez l'humain (Lewald, 2002; Chebat, et al., 2007; Anurova, et al., 2015; Maller, et al., 2016). Logiquement, cette période critique devrait se situer avant la fin du développement du

système visuel. Chez l'humain, le système visuel est presque complètement établi avant l'âge de 2 ans (Siu and Murphy, 2018). Chez toutes les espèces, la période critique se situe avant la fin de l'expansion corticale et de l'élagage synaptique.

V.3.2 Plasticité cérébrale dans les structures non-visuelles

Les résultats des analyses IRM ont montré qu'en plus de la plasticité dans les structures visuelles, il y a une plasticité dans plusieurs structures non visuelles. Une atrophie dans plusieurs régions cérébrales a été observée chez les souris aveugles incluant la partie postérieure du cortex cingulaire antérieur et la substance noire, des régions impliquées dans certaines fonctions visuelles. Cette atrophie, comme pour les structures visuelles, peut être expliquée par la désuétude.

D'autre part, une augmentation du volume a été mise en évidence dans différentes régions olfactives, dans le noyau central de l'amygdale ainsi que dans les corps mamillaires. Les corps mamillaires sont la terminaison des fibres fornicales, voies efférentes de l'hippocampe et jouent un rôle important dans la mémoire spatiale (Rosenstock, et al., 1977; Aggleton, et al., 1995). Les aveugles comptent sur leur mémoire spatiale pour la navigation et l'orientation au quotidien. En effet, les études chez l'humain ont montré que les aveugles ont une meilleure mémoire spatiale que les voyants (Cleaves and Royal, 1979; Postma, et al., 2007; Fortin, et al., 2008).

Les études sur la plasticité cérébrale chez l'humain ont montré que l'entraînement peut conduire à une augmentation du volume de la matière grise ainsi qu' à une augmentation de l'épaisseur corticale (Sluming, et al., 2002; Draganski, et al., 2004; Cannonieri, et al., 2007; Bermudez, et al., 2009; Foster and Zatorre, 2010; Wei, et al., 2011). Cette augmentation pourrait résulter d'une expansion de l'arborisation dendritique, une augmentation de la densité synaptique ou de la myélinisation (Zatorre, et al., 2012). Ceci expliquerait l'expansion de certaines structures non-visuelles chez les aveugles précoces. En effet, les aveugles comptent plus sur leurs modalités sensorielles subsistantes (Kupers and Ptito, 2014) ce qui entraîne une sollicitation plus importante des régions cérébrales concernées menant ainsi à leur expansion.

Des études ont réussi à établir un lien entre l'augmentation du volume de certaines aires corticales et des fonctions améliorées. Par exemple, chez les aveugles précoces, une meilleure navigation spatiale corrèle avec un volume plus grand de l'hippocampe (Fortin, et al., 2008) et

des meilleures performances olfactives corrèlent avec un volume plus grand des bulbes olfactifs (Rombaux, et al., 2010). Dans la présente étude, l'augmentation du volume dans les structures non visuelles pourrait aussi expliquer les changements comportementaux comme l'amélioration des performances olfactives accompagnée de plasticité dans les régions olfactives et l'hypersensibilité à la douleur accompagnée de plasticité dans l'amygdale observées chez les souris aveugles. D'autres études seraient nécessaires afin de déterminer les autres changements comportementaux découlant de la plasticité cérébrale observée dans les autres régions cérébrales chez les souris aveugles.

Chapitre VI Conclusions et perspectives

Il est bien établi que la cécité précoce entraîne des adaptations comportementales souvent accompagnées de changements structurels et fonctionnels dans le cerveau. Dans ce contexte, l'olfaction et la douleur sont les modalités sensorielles les moins investiguées. La souche nouvelle et unique ZRDBA, développée dans notre laboratoire, permet de comparer des groupes de souris voyantes et aveugles issues des mêmes portées et hébergées dans les mêmes cages. Ceci enlève le biais des différences comportementales et neuroanatomiques qui peuvent exister entre les différentes souches de souris et permet d'étudier les impacts directs de la cécité sur les modifications comportementales, fonctionnelles et neuroanatomiques.

Dans la présente thèse, nous avons examiné chez ce modèle de souris aveugle précoce les changements comportementaux olfactifs et nociceptifs ainsi que la plasticité cérébrale qui les accompagne. Nous avons également investigué les changements anatomiques, conséquents de la cécité, dans tout le cerveau chez ces souris ZRDBA.

Les résultats des études de cette thèse ont permis de mettre en évidence une amélioration des performances olfactives chez les souris aveugles qui est accompagnée d'une hypertrophie des bulbes olfactifs ainsi qu'une augmentation du volume dans d'autres structures impliquées dans la fonction olfactive (cortex piriforme, cortex orbitofrontal). Ces résultats permettent de faire le parallèle avec les résultats obtenus chez l'humain en olfaction et suggèrent la contribution de la plasticité cérébrale olfactive dans l'amélioration de fonction olfactive chez l'aveugle.

Nous avons aussi mis en évidence, une hypersensibilité à la douleur chez les souris aveugles qui est accompagnée d'une plasticité neuroanatomique dans l'amygdale et dans son noyau central. Ces résultats permettent d'expliquer, en partie, les mécanismes sous-jacents de l'hypersensibilité à la douleur observée chez les aveugles précoces et renforcent les données disponibles soulignant le rôle important de l'amygdale et du noyau central dans le traitement et modulation de la douleur.

Finalement, l'examen des changements morphométriques de tout le cerveau a montré une atrophie de la plupart des structures visuelles (cortex visuel primaire et secondaire et collicules supérieurs) ainsi qu'une hypertrophie d'autres structures non-visuelles telles que les

structures impliquées dans la fonction olfactive, la douleur et la navigation spatiale. Les résultats de cette thèse suggèrent que la plasticité cérébrale observée chez les aveugles précoces pourrait expliquer les changements comportementaux adaptatifs mis en évidence chez cette population. Ces résultats permettent de faire le parallèle avec les études réalisées chez l'humain et ouvrent la porte pour plus d'investigations des mécanismes sous-jacents de la plasticité cérébrale observée chez les aveugles.

Dans les prochaines études, il serait intéressant d'étudier la plasticité olfactive et nociceptive ainsi que la plasticité cérébrale sous-jacente chez un modèle de souris aveugles tardives (énucléation postnatale). Ceci permettrait de mettre en évidence l'importance de la période critique de développement dans la fonction olfactive et la douleur et de comparer les adaptations comportementales et cérébrales concernant ces modalités dans le cas de cécités précoce et tardive.

Afin de confirmer la contribution directe de l'amygdale dans l'hypersensibilité à la douleur, il serait également important d'utiliser des approches chimiogénétiques chez la même souche de souris. La chimiogénétique permettrait d'inhiber sélectivement le noyau central et/ou l'ensemble de l'amygdale et d'examiner son impact sur le comportement lié à la douleur chez les souris aveugles.

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Chapitre VIII Annexe

Article: Sympathetic regulation and anterior cingulate cortex volume are altered in a rat model of chronic back pain

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Abstract

Chronic pain is associated with autonomic disturbance. However, specific effects of chronic back pain on sympathetic regulation remain unknown. Chronic pain is also associated with structural changes in the anterior cingulate cortex (ACC), which may be linked to sympathetic dysregulation. The aim of this study was to determine whether sympathetic regulation and ACC surface and volume are affected in a rat model of chronic back pain, in which Complete Freund Adjuvant (CFA) is injected in back muscles. Sympathetic regulation was assessed with renal blood flow (RBF) changes induced by electrical stimulation of a hind paw, while ACC structure was examined by measuring cortical surface and volume. RBF changes and ACC volume were compared between control rats and rats injected with CFA in back muscles segmental (T10) to renal sympathetic innervation or not (T2). In rats with CFA, chronic inflammation was observed in the affected muscles in addition to increased nuclear factorkappa B (NF-kB) protein expression in corresponding spinal cord segments ($p=0.01$) as well as decreased ACC volume ($p<0.05$). In addition, intensity-dependent decreases in RBF during hind paw stimulation were attenuated by chronic pain at T2 ($p's<0.05$) and T10 ($p's<0.05$), but less so at T10 compared with T2 ($p's<0.05$). These results indicate that chronic back pain alters sympathetic functions through non-segmental mechanisms, possibly by altering descending regulatory pathways from ACC. Yet, segmental somato-sympathetic reflexes may compete with non-segmental processes depending on the back region affected by pain and according to the segmental organization of the sympathetic nervous system.

Introduction

Chronic pain is highly prevalent in the adult population, affecting approximately 20 % of individuals (Schopflocher, et al., 2011). In addition to pain and disability, chronic pain syndromes are associated with autonomic disturbance (Chelimsky, et al., 2001; Staud, 2008; Giannoccaro, et al., 2014; Chelimsky, et al., 2016). Consistent with these observations, animal and human studies on somato-autonomic reflexes show that nociceptive stimulation of the skin, muscles and joints can influence autonomic functions through somato-autonomic reflexes (Sato, et al., 1997; Desmarais, et al., 2011). However, the effects of chronic nociceptive inputs relevant for the physiopathology of chronic pain have been largely disregarded. Acute nociceptive stimulation of the limbs generally produces non-segmental sympathetic reflexes integrated in brain structures, while nociceptive stimulation of the trunk may evoke segmentally-organized sympathetic reflexes integrated in the spinal cord, in addition to non-segmental reflexes (A. Sato, 1971; Sato and Schmidt, 1973; R. Swenson, 1984; A. Kimura, 1995; A. Kimura, 1996; Budgell and Suzuki, 2000; Piché, et al., 2014). To our knowledge, only one study investigated somato-autonomic interactions in chronic conditions. In this study, nociceptive heat stimulation of a paw decreased skin temperature of the three non-stimulated paws. However, this response (vasoconstriction) was attenuated in rats with long-lasting hind paw hyperalgesia, reflecting sympathetic dysregulation (Vierck, et al., 2008). To our knowledge, however, the effects of longlasting nociceptive activity on sympathetic regulation in animal models of back pain have not been investigated. Considering the segmental organization of somato-sympathetic reflexes, chronic back pain may produce specific sympathetic dysregulation or an interaction of segmental and non-segmental sympathetic processes, which may be of clinical relevance.

Therefore, this interaction between chronic back pain and sympathetic regulation remains to be investigated. In addition to its effects on sympathetic functions, chronic pain can produce maladaptive plasticity in various brain regions (Johansen, et al., 2001; May, 2008; Zhuo, 2008; Blom, et al., 2014; Lu, et al., 2014; Newman, et al., 2014; Zhuo, 2014). For example, changes in brain structure and function in prefrontal, insular and cingulate areas have been observed in several studies and they may be associated with physiological or functional impairment (Apkarian, et al., 2004a; Apkarian, et al., 2004b; Schmidt-Wilcke, et al., 2006; Siddall, et al., 2006; Kuchinad, et al., 2007; Lloyd, et al., 2008; Metz, et al., 2009; Wand, et al., 2011; Saab,

2012; Piché, et al., 2013; Blom, et al., 2014). Among these regions, the anterior part of the cingulate cortex (ACC) is of particular interest in the study of autonomic dysregulation produced by chronic pain because it is involved in both pain and autonomic regulation (Mohr, et al., 2005; Vogt, 2005; Qu, et al., 2011). Indeed, ACC sends prominent projections to the somatosensory cortex, the amygdala, as well as to the periaqueductal gray matter (PAG) and spinal cord, structures that are involved in nociceptive and autonomic integration (Zhuo, 2014). In the rat, injection of complete Freund's adjuvant (CFA) in musculoskeletal tissues is a common model of chronic pain and may be performed in the hind paw (Walker, et al., 2003), tail (Vanegas and Schaible, 2004), joints (Masocha, 2013; Kaneguchi, et al., 2016), or muscles (Ambalavanar, et al., 2006; Chacur, et al., 2009; Asgar, et al., 2015). When injected in muscles or fascia, CFA induces chronic inflammation in the affected tissues, sensitization of dorsal horn neurons as well as neuroinflammatory changes in the corresponding spinal cord segments, including microglial activation (Chacur, et al., 2009; Hoheisel and Mense, 2015). Interleukine-6 (IL-6), nuclear factor-kappa B (NF-kB) and Cyclooxygenases-2 (COX-2) are three critical markers of the complex process of neuroinflammation. IL-6 is a potent neuroinflammatory signal known to stimulate microglia and astrocytes to release a cascade of proinflammatory cytokines and acute phase proteins (Luo and Zheng, 2016). NF-kB is a critical immediate early response gene involved in modulating cellular responses and apoptosis following diverse injuries (Snow and Albensi, 2016). COX-2, together with COX-1, is a key enzyme in the conversion of arachidonic acid into bioactive prostanoids, playing a central role in the inflammatory cascade (Patrignani and Patrono, 2015). In addition, COX-1 and COX-2 are actively involved in neuronal dysfunction induced by pro-inflammatory stimuli (Yagami, et al., 2016). Therefore, IL-6, NF-kB and COX-2 may contribute to sympathetic dysregulation and morphological changes in ACC that are associated with chronic back pain but this is still unknown. Considering the clinical implications of sympathetic dysregulation and ACC plasticity, this topic deserves further investigation.

The aim of the present study was to determine whether sympathetic regulation and ACC structure are altered in rats with chronic back pain. Sympathetic regulation was assessed with renal blood flow (RBF) changes induced by electrical stimulation of a hind paw, while ACC structure was examined by measuring the cortical surface and the volume. RBF changes and

ACC structure were compared between control rats and rats with chronic back pain in regions segmental (T10) or not (T2) to renal sympathetic innervation. In addition, protein expression of three known markers of neuroinflammation, namely NF-kB, COX-2 and IL-6, was measured by Western blot analysis of spinal cord tissues.

We hypothesized that somato-renal reflexes would be attenuated in rats with chronic back pain compared with controls. However, we expected differential effects for rats with chronic pain affecting the lower (T10) or upper (T2) thoracic spine, in accordance with the segmental organization of renal sympathetic innervation. As an indication of neuroinflammation associated with these pathological changes, we also anticipated increased protein expression of IL-6, NF-kB and COX-2 in corresponding spinal cord segments. Finally, we hypothesized that ACC volume would be decreased in rats with chronic pain.

Experimental procedures

Ethical approval

Experiments were performed on 42 male Wistar rats (body weight 300–450 g, Charles River Laboratories International, Willmington, MA, USA). Animals were kept in local facilities with a light/dark cycle of 14h/10h. All experimental procedures were approved by the animal care committee of "Université du Québec à Trois-Rivières", in accordance with the guidelines of the Canadian Council on Animal Care and the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP). Chronic pain model and animal groups Chronic back pain was induced by an intramusclar injection of Complete Freund adjuvant (CFA) (Difco Lab., Detroit, MI, USA), as described previously (Chacur, et al., 2009). Under ultra-short isoflurane anesthesia, a volume of 150 µL was injected through the skin in thoracic paraspinal muscles, using a 27-gauge needle. To examine the effect of chronic back pain on sympathetic regulation and brain morphology, experiments were conducted on 3 groups of rats. Rats from the control group (n=16) received a 150 µL isotonic saline injection in T10 paraspinal muscles. Rats from the two other groups received a 150 µL injection of CFA (Difco Lab., Detroit, MI, USA) in T10 (CFA-T10, n=16) or T2 (CFA-T2, n=10) paraspinal muscles. Twelve days post-injection, ten animals from each of the three groups were used for

the physiological experiment while six animals from the CFA-T10 group and the control group were used for the Western blot analyses. Animals used for the physiological experiments and the Western blot analyses are different because the former required perfusion of the whole body through the heart to extract rinsed and fixated muscles and brain, while the later required rapid extraction of the spinal cord in anesthetized animals without fixation. Also, only one group of rats with chronic pain was used for Western blot analyses (CFA-T10) because CFA was not expected to produce different spinal neuroinflammatory changes when injected in different regions of the back. The brain of 8 rats from the T2 (3) and T10 (5) CFA groups and 8 rats from the control group in which physiological experiments were conducted were also used to examine morphological changes induced by chronic pain in the cingulate cortex. Again, CFA was not expected to produce different morphological changes in ACC when injected in different regions of the back so no comparison was done between the T2 and T10 groups.

Assessment of neuroinflammation

Spinal cord extraction

For Western blot analyses, rats were deeply anesthetized with urethane (1.6 g.kg-1 i.p.) on day 12 and surgical procedures were initiated to extract the spinal cord. In a prone position, the vertebral canal was opened and the thoracolumbar spinal cord was extracted from T6 to T13. The spinal cord was then immediately frozen at – 80°C to be later processed. Rats were then killed with lethal dose of urethane (2.0 g.kg-1 i.p.).

Electrophoresis and immunoblot analyses

Total proteins from spinal cord tissues (control group, n=6; CFA-T10, n=6) were extracted with Tri-Reagent (Sigma-Aldrich, St. Louis, MO, USA) and a protease inhibitor mix (Active Motif, Brockville, ON, Canada) was then added to avoid protein digestion. Protein concentrations were measured with a bicinchoninic acid protein assay kit (Pierce Biotechnology Inc., Rockford, IL), and equal amounts were loaded onto 12% sodium dodecyl sulfate (SDS) polyacrylamide gels. After electrophoretic separation (125 V, for 1.5 h), gels were transferred onto polyvinylidene difluoride (PVDF) membranes (0.22 μm pore size, BioRad, Hercules, CA), at 25 V overnight. The membranes were blocked for 1 h at room temperature into Blotto B (1%

non-fat powdered milk, 1% bovine serum albumin [BSA], 0.05% Tween 20, 0.5 mg/mL sodium azide, in Tris buffered saline) and incubated overnight at 4°C with primary antibodies anti-COX-2 (1:50), anti-NFkB (1:50), anti-IL-6 (1:100) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and anti-β-actin (1:300) (Sigma-Aldrich, St. Louis, MO, USA), as internal control. The blots were then washed and incubated with peroxidase-conjugated secondary antibody (1:2000) in Blotto B for 1 hour at room temperature and finally, developed with an enhanced chemiluminescence substrate solution (Thermo Scientific). Immunopositive signals were visualized with the AlphaEase FC imaging system. Analyses were performed with AlphaEase FC software (Alpha Innotech, San Leandro, CA) and ImageJ (imagej.nih.gov) software.

Physiological experiment

Surgical procedures

Surgical procedures were initiated after animals were deeply anesthetized with urethane (1.2 g.kg-1 i.p.). The right jugular vein was catheterized and additional urethane doses were administered as needed to maintain the depth of anesthesia by bolus i.v. injections (approximately 10% of initial dose). In addition to stable systemic arterial blood pressure, the depth of anesthesia was routinely confirmed by the absence of withdrawal reflexes (paw pinching). Systemic mean arterial pressure (MAP) was recorded continuously with a sampling of 1000 Hz (Spike 2, Cambridge Electronic Design, Cambridge, UK) from a canula inserted into the right common carotid artery and connected to a pressure transducer (Harvard Apparatus, Holliston, MA, USA). Animals were artificially ventilated using a tracheal canula (SAR-830/P Ventilator, CWE inc., Ardmore, PA, USA) and end-tidal CO2 concentration was maintained to approximately 3.5% by controlling respiratory rate and tidal volume (CAPSTAR-100 Carbon dioxyde analyser, CWE inc., Ardmore, PA, USA). Body temperature was monitored with a rectal probe and was maintained at 37.5±0.5 °C with a body temperature control system (TCAT-2LV controller, Physitemp instruments inc, USA).

Electrical stimulation

Electrical stimulation of the hind paw (trains of 400 pulses of 1-ms duration at 20Hz) was delivered using a train generator (Grass Medical instruments, model S88, Quincy, MA,

USA) and a constant-current stimulator (Model DS7, Digitimer Ltd, Welwyn Garden city,UK) with a pair of subdermal needle electrodes (Model E2, Grass Medical instruments, Quincy, MA, USA) inserted on the lateral aspect of the fourth and fifth digits. Once surgical procedures were completed, a stimulus-response curve of RBF changes was obtained with 8 different intensities (0.5, 1, 1.5, 2, 5, 10, 20, 30 mA) delivered with an inter-stimulus interval of 180 s, during which RBF and MAP recovered to baseline.

Recording of renal blood flow

The animal was placed in a prone position, with the left kidney exposed. The kidney was covered with warm paraffin oil in a pool made of sutured skin flaps. RBF was measured using a laser Doppler probe (Micro-needle probe TSD145, Biopac systems, Goleta, CA, USA) gently placed on the left kidney surface. RBF signal was sampled at 120 Hz with a Power1401 acquisition system (Cambridge Electronic Design, Cambridge, UK) and recorded for offline analysis (Spike 2, Cambridge Electronic Design, Cambridge, UK). RBF signal was filtered offline with a time constant of 3 seconds (Spike 2, Cambridge Electronic Design, Cambridge, UK).

Muscle histology

At the end of the physiological experiment, rats (n=30) were perfused through the heart with a 10% formaline solution. Thoracic paraspinal muscles were removed bilaterally. Tissues were dehydrated, cleared and embedded in paraffin (tissue processor, Tissue Tek VIP E300, Sakura Finetek, Torrance, CA, USA). Serial sections of 5 μm thickness were then cut using a rotary microtome (Reichert Histostat microtome model 820, Reichert Technologies, Depew, NY, USA) and were mounted onto adhesive coated slides. Finally, sections were stained with a hematoxylin-erythrosine-saffron coloration (Autostainer XL, Leica microsystems, Wetzlar, Germany) for histological examination under a microscope to detect the presence of chronic inflammation, as described earlier (Chacur, et al., 2009). One rat from the CFA-T10 group did not show any evidence of chronic inflammation and was excluded from all analyses.

Brain morphometry

Following perfusion through the heart with 100 mL of saline and 500 mL of a 10% formalin solution, brains were collected and post-fixed in formalin for at least 24 h. After cryoprotection with a 30% sucrose solution for 48 hours at 4°C, coronal sections of 50 µm thickness were collected using a freezing microtome (Leica VT1000S, Leica microsystems, Wetzlar, Germany), rinsed in PBS and mounted on gelatine-coated microscope slides. They were then processed for Nissl staining, dehydrated and coverslipped. Brain sections were analyzed under an Olympus BX50W1 microscope coupled to a CCD camera (Optronix, MicroBrightField, Williston, VT, U.S.A.). Delineation of cingulate cortex areas (ACC and MCC) was performed with the Neurolucida Software (MicroBrightField, Williston, VT, U.S.A.) based on Caviness cytoarchitectonic description (Caviness, 1975). The cortical surface of each area was calculated by multiplying the length of a line drawn along the pial surface by the spacing between the measured sections (100µm). The volume was calculated by multiplying the sectional surface by the antero-posterior span of the cortical area. The A-P coordinates were based on Paxinos and Watson (1986) (ACC: B +2.76 to B -0.36; MCC: B-0.48 to B-1.56). The experimenter was blinded for all analyses to avoid any bias in group comparisons.

Data analyses

For the physiological experiment, RBF and MAP were analysed with Spike 2 (Cambridge Electronic Design, Cambridge, UK). The onset-to-peak amplitude of shockevoked RBF and MAP changes was extracted to quantify the response to each shock train and each value was normalized as percent change relative to the mean signal value during a 30-s baseline window. These values were averaged across animals for each intensity and mean responses were compared between groups.

Statistical analyses

Statistical analyses were performed with Statistica v13.0 (Dell Inc., Tulsa, OK, USA). All data are expressed as mean ± S.E.M. and the significance threshold was set to $p \leq 0.05$ (two-tailed) for all analyses. Distribution was assessed with the KolmogorovSmirnov test and

distribution normality was confirmed for all variables. For neuroinflammatory changes, COX-2, NF-kB and IL-6 protein expression was compared between groups (CFA-T10 vs controls) using two-sample independent t-tests. For ACC and MCC, the cortical surface and the volume were compared between groups using twosample independent t-tests. For the physiological experiment, shock-evoked RBF and MAP changes were compared between intensities and groups using Greenhouse-Geisser corrected mixed-model ANOVA. Significant effects were decomposed using the Fisher post-hoc test.

Results

Chronic muscle inflammation Muscle sections were examined to confirm the presence or absence of inflammatory changes. Leukocyte infiltration, mainly macrophagic and lymphoid cells, were observed in rats from the chronic pain groups. No sign of inflammation was observed in any of the control rats. Representative examples of muscle sections from one control rat and one rat with chronic pain are shown in Figure 1.

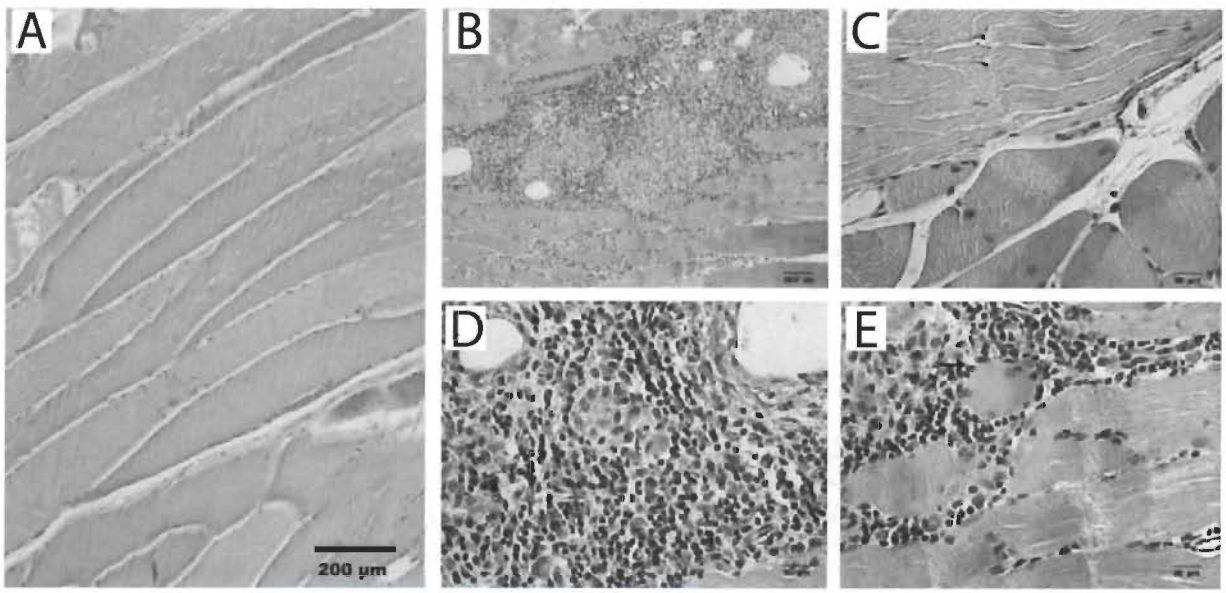


Fig. 1. Chronic muscle inflammation. Representative examples of back muscles from animals with chronic pain and controls. (A) Normal back muscle from a control rat 12 days after a vehicle saline injection. (B–E) Inflamed back muscles from a rat with chronic pain 12 days after a CFA injection. (B) Macrophagic and lymphoid cell infiltration. (C) Muscle fiber

atrophy. (D) Giant cell among macrophages and lymphocytes. (E) Fiber regeneration with myoblasts.

Spinal cord neuroinflammation

Protein expression of IL-6, NF-kB and COX-2 were compared between spinal cords of rats with chronic pain (CFA) and controls using two-sample independent t-tests. Rats with chronic pain (CFA) showed a significant increase of protein expression for NFkB ($t10=3.1$, $p=0.01$), but no significant change for protein expression of IL-6 ($t10=0.7$, $p=0.5$) or COX-2 ($t10=1.1$, $p=0.3$) compared with controls (see Figure 2).

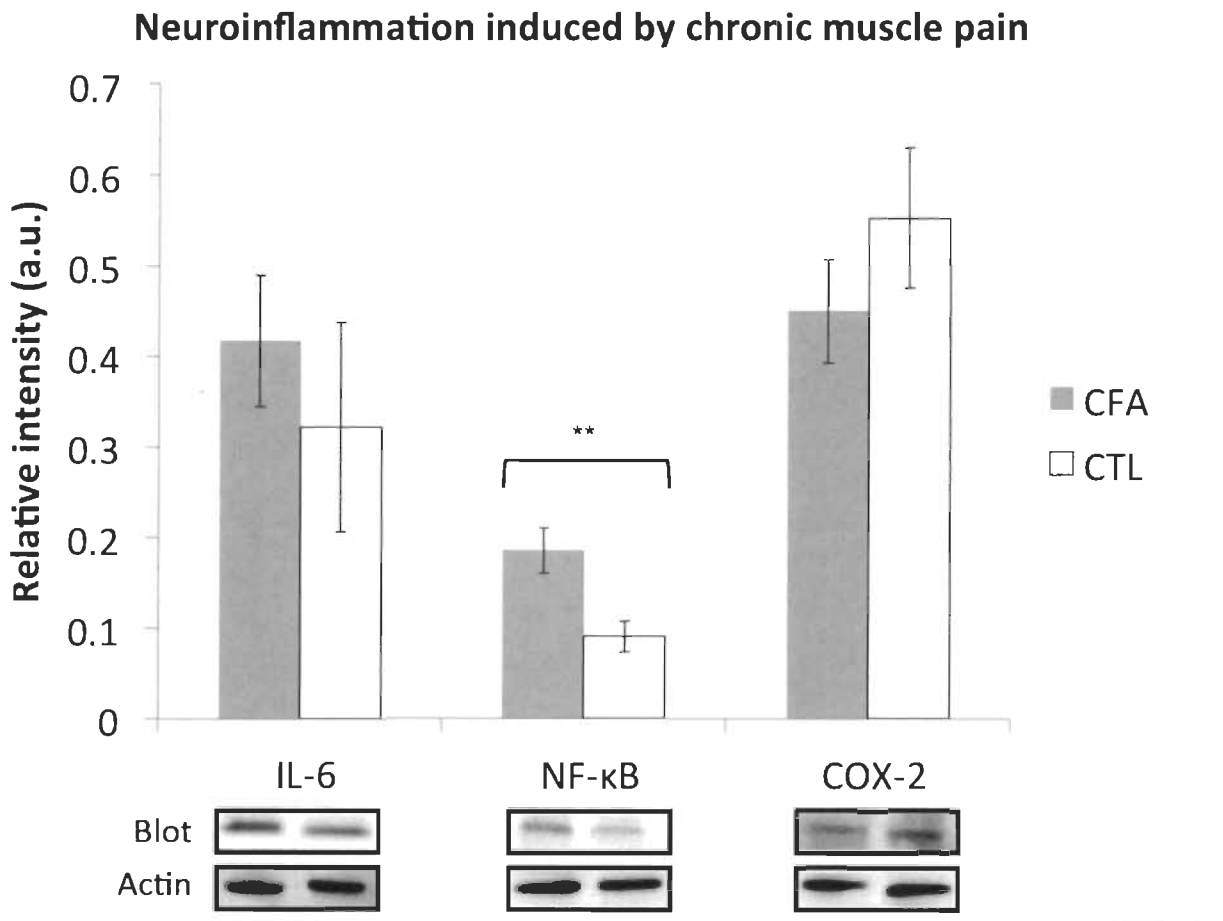


Fig. 2. Neuroinflammatory changes in the spinal cord. IL-6, NF-kB (NKB) and COX-2 protein expression in rats with chronic pain and controls. Chronic pain increased the protein expression of NF-kB but not IL-6 and COX-2 in the CFA-T10 group compared with

the control group. *p < 0.05. Western blots bands are representative samples of at least four different experiments. b-actin protein expression was used to normalize IL-6, NKB and COX-2 protein expression, therefore reported in the histogram as a relative values (a. u. = arbitrary units).

Changes in brain structure induced by chronic pain

The cortical surface and volume of ACC and MCC were compared between groups using two-sample independent t-tests (see Figure 3). ACC volume was decreased in rats with chronic pain compared with controls for the left and right hemispheres ($t_{14}=2.1$, $p=0.056$ and $t_{14}=2.2$, $p=0.048$, respectively) and for both hemispheres combined ($t_{14}=2.1$, $p=0.049$). No significant difference was observed between groups for ACC surface for the left or right hemispheres or for both hemispheres combined ($t_{14}=1.1$, $p=0.29$; $t_{14}=1.3$, $p=0.22$, and $t_{14}=1.2$, $p=0.25$, respectively). As for MCC, no significant difference in volume was observed between groups for the left or right hemispheres or for both hemispheres combined ($t_{14}=0.02$, $p=0.98$; $t_{14}=0.01$, $p=0.99$; $t_{14}=0.01$, $p=0.99$). Also, no significant difference was observed between groups for MCC surface for the left or right hemispheres or for both hemispheres combined $t_{14}=0.68$, $p=0.51$; $t_{14}=0.46$, $p=0.65$; $t_{14}=0.57$, $p=0.58$).

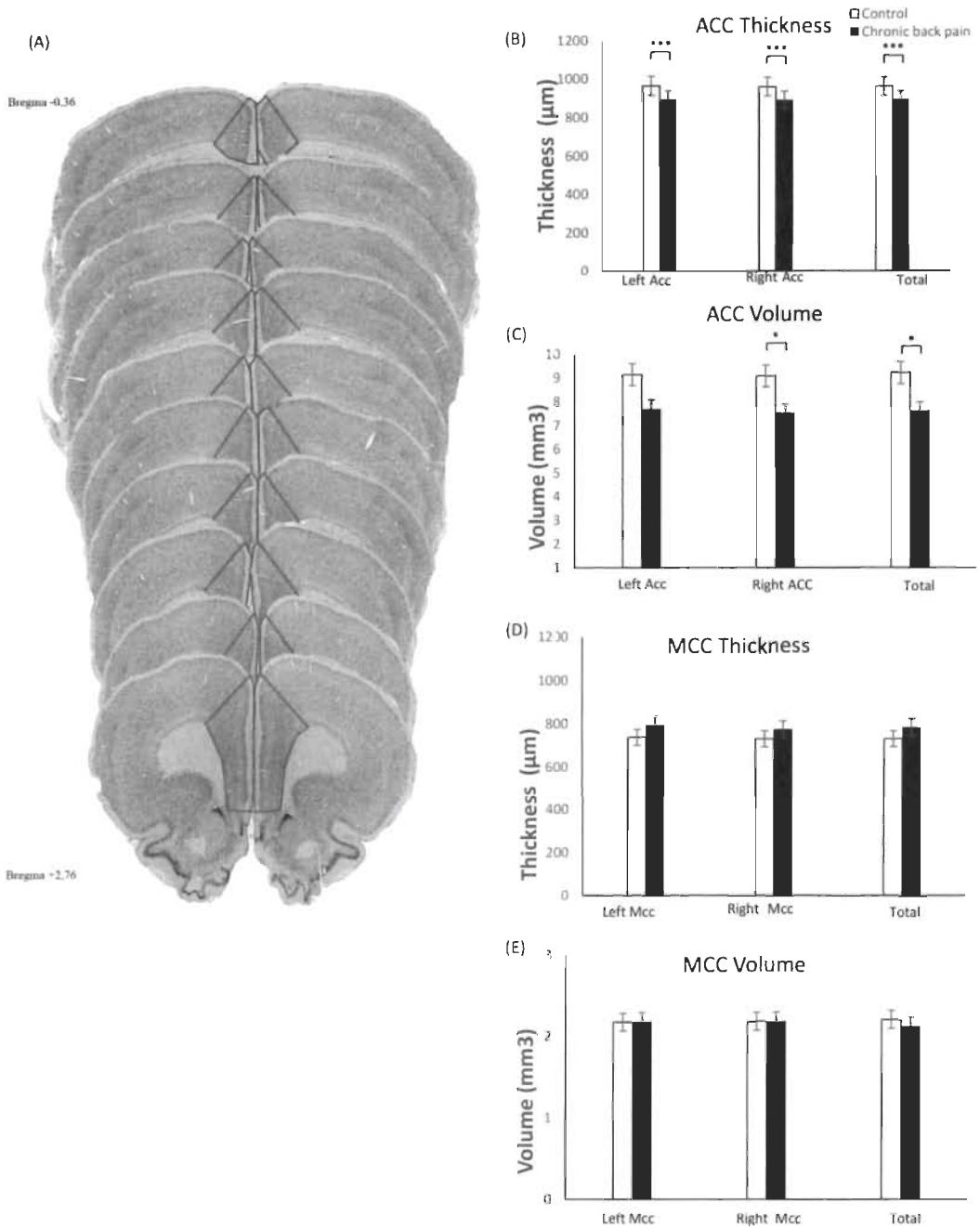


Fig. 3. Cingulate cortex structure. (A) Delineation of ACC area (B) ACC volume was significantly decreased compared with controls for the left hemisphere, right hemisphere and both hemispheres combined. (C) No significant difference was observed for the left hemisphere, right hemisphere and both hemispheres combined for ACC surface. (D, E) MCC volume and surface were not significantly different between groups for the left or right hemisphere or for both hemispheres combined. *$p < 0.05$. #$p = 0.056$.

Sympathetic regulation

Electrical stimulation of the hind paw produced a robust decrease of RBF mirrored by a robust increase in MAP (see individual example in Figure 4). These RBF and MAP changes were compared between groups for each intensity by a mixed model ANOVA (see Figure 5). RBF decreases were marginally different between groups (main effect: $F_{2,26}=3.3$, $p=0.054$) and showed a strong intensity-dependent response (main effect: $F_{7,182}=69.3$, $p<0.01$). Moreover, the intensity-dependant decrease in RBF was significantly different between groups (interaction: $F_{14,182}=2.8$; $p<0.01$, $p<0.001$ and $p<0.001$, respectively). A similar but weaker attenuation was observed for the CFA-T10 group compared with the control group and only for the 20 and 30 mA intensities (both $p<0.05$) and tended to be greater for the 30 mA intensity ($p=0.078$).

Besides, MAP increases showed a significant intensity-dependent effect (main effect: $F_{7,182}=133.2$, $p<0.001$) but were not significantly different between groups (main effect: $F_{2,26}=2.6$, $p=0.10$). Moreover, MAP changes was not significantly different between groups across intensities (interaction: $F_{14,182}=1.5$, $p=0.22$).

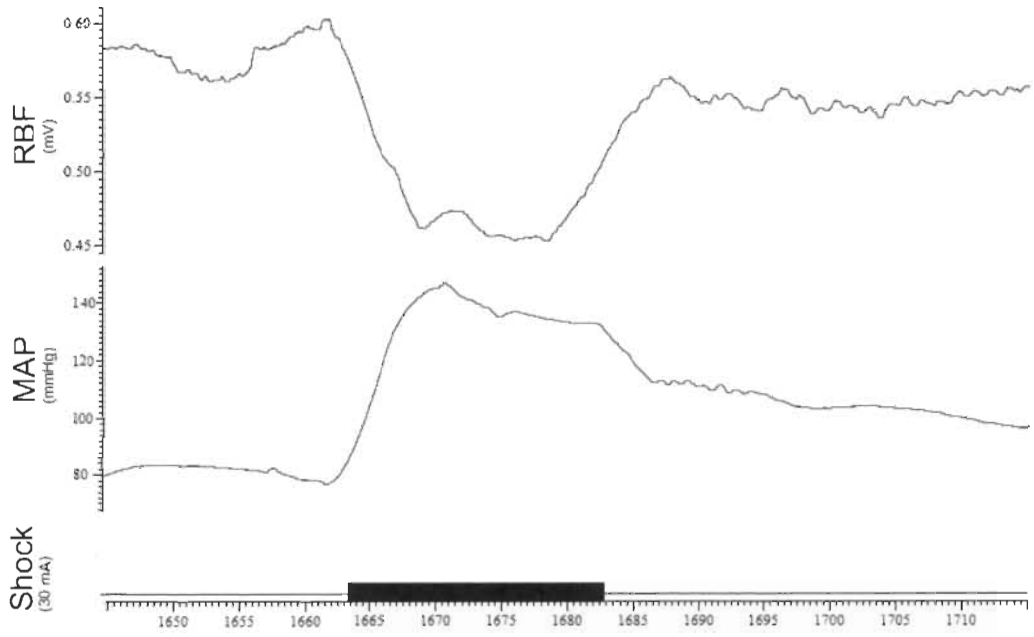
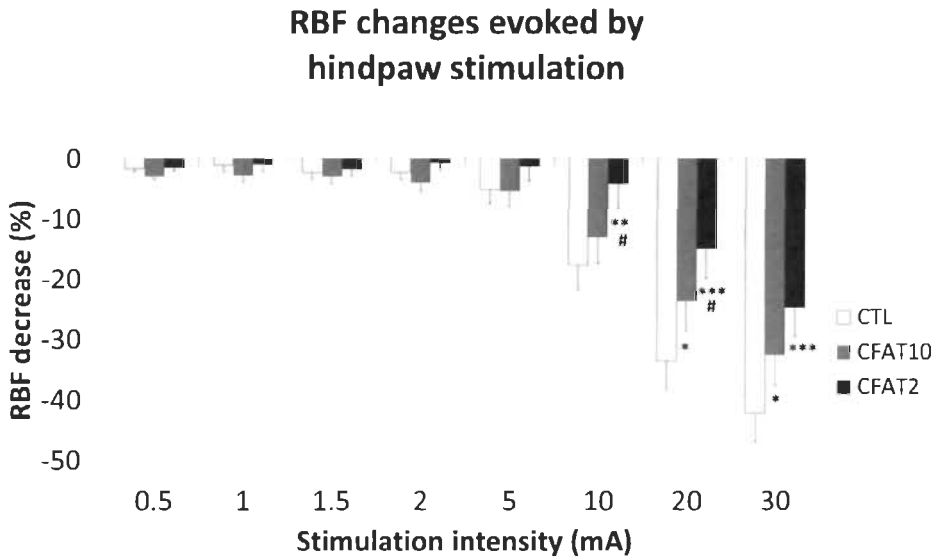


Fig. 4. Individual examples of recordings. The traces represent RBF and MAP recordings, respectively. Electrical stimulation of the hind paw produced a robust decrease of RBF mirrored by a robust increase in MAP (see illustration of mean responses for each group in Fig. 5).



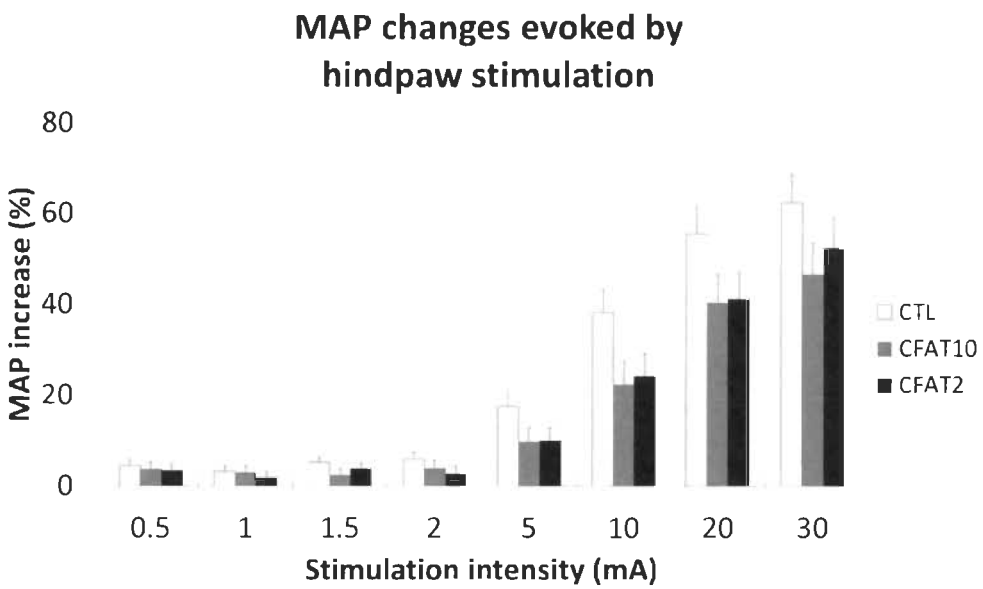


Fig. 5. Sympathetic dysregulation induced by chronic pain Mean responses to electrical stimulation at various intensities. (A) RBF decreases were significantly attenuated in the CFA-T2 group compared with the control group for 10-, 20- and 30-mA intensities. Significant but weaker attenuation was observed in the CFA-T10 compared with the control group for 20 and 30 mA intensities. RBF attenuation was significantly greater in the CFA-T2

group compared with the CFA-T10 group for the 10-mA and 20-mA intensities. (B) MAP changes were not significantly different between groups across intensities.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared with the control group. #$p < 0.05$ compared with the CFAT10 group.

Discussion

This is the first study to examine the effects of chronic back pain on the regulation of somato-sympathetic reflexes (shock-evoked RBF decreases) in rats. Results indicate that RBF decreases were attenuated by chronic pain but this effect was different depending on the back region affected by pain, with weaker effects for pain in the back region which nociceptive afferents project to spinal segments providing renal sympathetic innervation. Chronic back pain also decreased ACC volume and produced neuroinflammatory changes in the spinal cord, as indicated by increased NF-kB protein expression. Altogether, these results show that chronic back pain alters sympathetic regulation through non-segmental mechanisms, possibly by altering descending regulatory pathways from ACC. However, segmental somato-sympathetic reflexes may compete with non-segmental processes depending on the back region affected by pain and according to the segmental organization of the sympathetic innervation.

Dysregulation of sympathetic reflexes by chronic pain

Nociceptive simulation of a hind paw produces sympathetic responses involving reflex arcs comprising ascending pathways from the spinal cord to the brain and descending pathways from the brain back to the spinal cord (Sato, et al., 1997). In the present study, this was observed as decreased RBF, due to increases in renal sympathetic nerve activity as reported earlier (Johns, et al., 2011). This RBF response, however, was attenuated by chronic pain. Moreover, the attenuation was different depending on the back region affected by pain, with weaker effects for pain at T10 compared with T2. In the rat, the kidney receives sympathetic innervation from the lower thoracic segments, while the upper thoracic segments provide no contribution (Strack, et al., 1988). This suggests that chronic nociceptive inputs from a back area could modulate the supraspinally-mediated sympathetic reflex, through a segmental or non-segmental mechanism.

Furthermore, the present results indicate that the segmental inputs from T10 competes with the non-segmental attenuation of sympathetic reflexes by chronic pain, which was evidenced here by weaker attenuation of the RBF response. In a previous study in the rat, long-lasting hyperalgesia on the dorsal hind paw was associated with sympathetic dysregulation (Vierck, et al., 2008). Consistent with the present results, the sympathetic response to nociceptive somatic stimulation was attenuated. Based on a previous study, this attenuation of phasic vasoconstriction is indicative of increased tonic sympathetic activity (Cooke, et al., 1990). Accordingly, the competing effects of the segmental mechanism in the present study would decrease tonic renal sympathetic activity. To our knowledge, the only study investigating the effect of nociceptive stimulation of the spine on autonomic regulation of renal functions examined how mechanical stimulation of the spine affects renal sympathetic nerve activity (Sato and Swenson, 1984). In this study, renal sympathetic nerve activity was decreased by mechanical stimulation of the thoracolumbar spine. Although this inhibitory response was suggested to be mediated at supraspinal levels, the same stimulation produced the opposite response after spinalization. Accordingly, chronic pain at T10 in the present study may have different effects on renal sympathetic activity compared with chronic pain at T2, which provides no segmental innervation of the kidney. This may occur through alteration of sympathetic preganglionic neurons innervating the kidney. This issue remains to be further investigated but the present findings provide an interesting avenue to investigate how chronic back pain may affect specific functions of the autonomic nervous system.

Neuroinflammation and chronic pain

Previous studies showed that intramuscular CFA results in sensitization of dorsal horn neurons in a rat model of back pain (Hoheisel and Mense, 2015) as well as spinal cord neuroinflammation in a rat model of monoarthritis (Chen, et al., 2015). Neuroinflammation is characterized by infiltration of immune cells, activation of microglia, and secretion of inflammatory mediators in the central nervous system (Marchand, et al., 2005; Chen, et al., 2015) and plays an important role in inducing and sustaining chronic pain (Ma and Zhao, 2002; Chacur, et al., 2009). In the present study, the expression of neuroinflammatory proteins IL-6 and COX-2 was not significantly increased in rats with chronic pain compared with controls.

This may indicate that neuroinflammation was not fully developed or that these markers should be detected earlier in this particular rat model of chronic pain. Nevertheless, the present results are generally consistent with previous findings. For instance, only slight increases in IL-6 were observe in a rat model of inflammatory pain, which seems to play a less important role in pain chronification, at least of inflammatory origin (Arruda, et al., 1998; Lee, et al., 2010). The present results are also consistent with the lack of change in COX-2 mRNA or protein expression in the spinal cord in animals with surgical inflammation (Dolan, et al., 2003). This does not exclude the role of COX-2 in pain chronification. Indeed, COX-2 induction is a rapid and transient event. In rats with peripheral inflammation induced by CFA, its expression in the spinal cord is downregulated within 3 days (F. Beiche 1998), which is consistent with the present results obtained 12 days following CFA injection. In contrast to these findings, increased expression of the NF-kB protein was observed in the CFA rats. NF-kB is implicated in the up-regulation of proinflammatory mediators (L.A.J O'Neilla, 1997; Sha, 1998; Yamamoto and Gaynor, 2001). NF-kB is also associated in neurobiological functions related to chronic pain such as neurodegeneration and apoptosis (Costigan, et al., 2009) and is regulated by the same signalling pathways that induce synaptic plasticity (Meffert, et al., 2003; Boersma and Meffert, 2008). Thus, it might play an important role in pain chronification, as in the present study. The specific increase in protein expression of NF-kB after 12 days of CFA treatment deserves further evaluation considering that neuroinflammation is sometimes the cause of irreversible loss, but it may also be where the solution stands to restore homeostasis (Renaud, et al., 2015).

Changes in the anterior cingulate cortex

volume In the present study, chronic back pain resulted in decreased ACC volume. This is consistent with the decrease in ACC volume in rats with chronic neuropathic pain, although these changes were observed after about 20 weeks (Seminowicz, et al., 2009). To our knowledge, no other studies on chronic pain models have reported structural changes in the brain. Therefore, the present results are novel and extend previous findings from the Seminowicz study by showing that even after 12 days of pain associated with chronic peripheral and spinal inflammation, brain structure can be altered. The results are also relevant from a transaltional point of view. Indeed, they are consistent with several studies showing structural

changes of ACC in patients with chronic pain, including decreased gray matter density in patients with fibromyalgia (Kuchinad, et al., 2007) and decreased gray matter volume in patients with chronic osteoarthrisis, (Rodriguez-Raecke, et al., 2009). Altogether, these findings suggest that chronic pain could produce cortical atrophy, although the mechanisms of these changes remain unclear (May, 2008). One possibility would be the activation of microglia that produces neurodegeneration and apoptosis (Costigan, et al., 2009). Consistent with this interpretation, glial cells were observed in the brain of patients with chronic back pain (Loggia, et al., 2015). An important finding of the present study is the volume changes in ACC in the same animals that show sympathetic dysregulation. Although we cannot establish a causal link between these two observations, it is possible that the atrophy of ACC may have contributed to the attenuation of sympathetic responses in rats with chronic pain. Accordingly, the ACC is involved in autonomic functions and more specifically in the regulation of the sympathetic activity (Critchley, et al., 2003; Luu and Posner, 2003; Critchley, et al., 2005). Consistent with this, electrical stimulation of the ACC can elicit autonomic responses in animals (kaada, 1949; A Burns, 1985) and patients with ACC damage fail to generate appropriate sympathetic responses (Critchley, et al., 2003). The ACC is functionally connected to the PAG, which is then connected to the rostral ventromedial medulla (RVM), forming the ACC–PAG–RVM network involved in pain modulation (Kong, et al., 2010). The RVM also projects to spinal cord neurons to regulate autonomic functions, through sympathetic preganglionic neurons (Mason, 2005). This supports the possibility that ACC atrophy could lead to sympathetic dysregulation, as observed in the present study. Further investigations are needed in order to understand the exact mechanisms underlying this process but the present findings support the link between central processes involved in pain chronification and sympathetic dysregulation.

Conclusion

Altogether, the present results show that chronic back pain alters sympathetic regulation through non-segmental mechanisms, possibly by altering descending regulatory pathways from ACC. However, segmental somato-sympathetic reflexes may compete with non-segmental processes when pain affects somatic tissues which nociceptive afferents also provide organ-

specific sympathetic innervation. It remains to be determined how neuroinflammation contribute to these pathological changes, both in the spinal cord and brain.

COMPETING INTERESTS

The authors declare no competing interests and no relationship that may lead to any conflict of interest.

AUTHOR CONTRIBUTIONS

Sara Touj, Dr. Sébastien Houle and Dr. Renaud Jeffrey Gauthier contributed to data collection, analyses and interpretation as well as manuscript writing. Professor Djamel Ramla contributed to the histopathological aspects of the study. Dr. Harumi Hotta and Professor Gilles Bronchti contributed to data analyses and interpretation as well as manuscript writing. Mathieu Piché and Maria-Grazia Martinoli contributed to all aspects of the research and obtained funding to conduct the study. Each author read and approved the final version of the manuscript.

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Abbreviations

ACC: anterior cingulate cortex; ANOVA: analysis of variance; CFA: complete Freund adjuvant; CTL: control; DLPFC: dorsolateral prefrontal cortex; IASP: International Association for the Study of Pain; IC: insular cortex; LTP: long-term potentiation; MAP: mean arterial pressure; MCC: midcingulate cortex; mPFC: median prefrontal cortex; NFkB: nuclear factor-kappa B; PAG: periacqueductal grey matter; RBF: renal blood flow: RVM: rostral ventromedial medulla; SEM: standard error of the mean; T2, T6, T10, T13: thoracic segment 2, 6, 10, 13.

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