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Etude du cerveau en développement et de ses adaptations face aux situations critiques

G. Loron

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G. Loron. Etude du cerveau en développement et de ses adaptations face aux situations critiques. Tissues and Organs [q-bio.TO]. Université de Reims Champagne Ardenne, 2022. tel-03940115

HAL Id: tel-03940115

<https://hal.science/tel-03940115>

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Mémoire

En vue de l'obtention de l'

HABILITATION A DIRIGER DES RECHERCHES

Soutenue publiquement le 22 novembre 2022



Gauthier Loron
MCU-PH Physiologie
CReSTIC EA3804



Devant le jury composé de
Madame le Professeur Nathalie Bednarek
Madame le Professeur Frédérique Bonnet-Brilhault
Monsieur le Professeur Pierre Burbaud
Monsieur le Professeur Stéphane Marret
Monsieur le Professeur Pierre Mauran
Monsieur le Professeur Fabrice Wallois

Ecole Doctorale Sciences Fondamentales Santé

1	<u>CURRICULUM VITAE</u>	5
1.1	ETAT CIVIL	5
1.2	PRESENTATION	5
1.3	CURSUS UNIVERSITAIRE	6
1.4	DIPLOMES UNIVERSITAIRES ET AUTRES FORMATIONS	6
1.5	TITRES HOSPITALIERS	7
1.6	MOBILITES	7
1.7	INSCRIPTION A DES SOCIETES SAVANTES	8
1.8	RESPONSABILITES LOCALES, REGIONALES, NATIONALES	8
1.9	REVUE D'ARTICLES	8
1.10	PRIX, BOURSES, FINANCEMENTS	8
2	<u>RESUME DU PROJET HOSPITALIER</u>	9
2.1	SERVICE DE MEDECINE NEONATALE ET REANIMATION PEDIATRIQUE	9
2.2	CONSULTATIONS	9
2.3	ÉLECTROPHYSIOLOGIE	9
2.4	PROJET HOSPITALIER	10
3	<u>ENSEIGNEMENT</u>	11
3.1	SYNOPSIS	11
3.2	FORMATION A L'ENSEIGNEMENT	11
3.3	RESPONSABILITE ADMINISTRATIVE D'ENSEIGNEMENT	11
3.4	ENSEIGNEMENTS DE LA PHYSIOLOGIE	11
3.5	ACTIVITES D'ENSEIGNEMENT HORS PHYSIOLOGIE.	12
3.5.1	DES/DESC, DIU, DEUXIEME CYCLE DES ETUDES MEDICALES	12
3.5.2	ENSEIGNEMENT PAR SIMULATION	12
3.6	ENSEIGNEMENTS ANTERIEURS	12
3.7	PROJET D'ENSEIGNEMENT	13
3.7.1	PROMOUVOIR LA PHYSIOLOGIE MEDICALE A L'UNIVERSITE DE REIMS	13
3.7.2	ÉVOLUER AVEC LES NOUVELLES TECHNIQUES PEDAGOGIQUES	13
4	<u>PARCOURS EN RECHERCHE DE 2007 A 2022</u>	15
4.1	ACTIVITE DE RECHERCHE PREDOCTORALE	15
4.2	MASTER II ET DOCTORAT	17
4.2.1	MASTER II	17
4.2.2	THESE D'UNIVERSITE : ETUDE DES EFFETS CEREBRAUX DU MONOXYDE D'AZOTE INHALE CHEZ LE RATON NOUVEAU-NE	17
4.2.3	INFLAMMATION MENINGEE ET EFFET IMMUN-MODULATEUR D'UNE QUINOLONE	20
4.3	ACTIVITE DE RECHERCHE POST-DOCTORALE	20
4.3.1	TRAVAUX SUR L'EEG D'AMPLITUDE	20
4.3.2	ABAQUES DE VELOCITE DOPPLER CHEZ LE GRAND PREMATURE	23
4.3.3	ANALYSE MORPHOLOGIQUE : ECHOGRAPHIE ET IRM CEREBRALES	24
4.3.4	INVESTIGATIONS MORPHOLOGIQUES ET ELECTROPHYSIOLOGIQUES DANS L'ENCEPHALOPATHIE ANOXO-ISCHEMIQUE	27

ACTIVITES D'ENCADREMENT	29
4.4 THESE D'UNIVERSITE	29
4.5 THESE D'EXERCICE POUR LE DOCTORAT DE MEDECINE	29
4.6 ENCADREMENT DE MASTERE 2 RECHERCHE	29
4.7 MEMOIRE DE DES DE PEDIATRIE	29
4.8 MEMOIRE DE DIU	29
5 PUBLICATIONS ET COMMUNICATIONS	30
5.1 SYNOPSIS	30
5.2 PLAN DE PUBLICATION	31
5.2.1 ARTICLES ACCEPTES, EN COURS DE PUBLICATION	31
5.2.2 ARTICLES SOUMIS EN COURS DE REVUE	31
5.2.3 ECRITURE EN COURS	31
5.3 PUBLICATIONS	31
5.4 COMMUNICATIONS ORALES INTERNATIONALES	35
5.5 POSTERS AFFICHES LORS DE CONGRES INTERNATIONAUX	36
5.6 COMMUNICATIONS ORALES NATIONALES	36
5.7 CONFERENCES INVITEES	38
6 PROJET DE RECHERCHE	39
6.1 STRUCTURE DE RECHERCHE DE RATTACHEMENT	39
6.2 THEME DU PROJET DE RECHERCHE	39
6.3 SUIVRE LES ALTERATIONS STRUCTURELLES DANS UN CERVEAU EN DEVELOPPEMENT	41
6.3.1 ALGORITHME DE SEGMENTATION AUTOMATIQUE	41
6.3.2 SEGMENTATION CEREBRALE ET DEVENIR	43
6.3.3 UTILISATION DE L'ALGORITHME DE SEGMENTATION AU-DELA DE LA COHORTE EPIRMEX	43
6.3.4 TRAITEMENT D'IMAGES ECHOGRAPHIQUES	43
6.4 DEVELOPPEMENT DES OUTILS ELECTROPHYSIOLOGIQUES : LA SITUATION DE L'ANOXO-ISCHEMIE A TERME.	45
6.4.1 VERIFIER LA CONCORDANCE DES INTERPRETATIONS AEEG ENTRE EXPERTS ET NON-EXPERTS	45
6.4.2 DRESSER LE PANORAMA DE L'UTILISATION DE L'EEG EN FRANCE DANS L'ANOXO-ISCHEMIE	45
6.4.3 EXPLOITATION DES RELECTURES EXPERTES	46
6.4.4 VERS UNE AIDE A L'INTERPRETATION EN TEMPS REEL DE L'EEG	47
6.5 ÉVALUER L'APPORT DU MONITORAGE DANS LA PRISE EN CHARGE DU PATIENT	47
6.6 PROMOUVOIR LES MECANISMES D'ADAPTATION ET DE PLASTICITE : PHRCI CALIN	48
6.6.1 RATIONNEL DE L'ETUDE	48
6.6.2 METHODOLOGIE DE L'ETUDE	49
6.6.3 RESULTATS ATTENDUS ET PERSPECTIVES :	51
7 CONCLUSION	53
8 BIBLIOGRAPHIE	54
9 ANNEXES	59

Préambule

Madame, Monsieur, Chers Maîtres,

Ce mémoire présente successivement un curriculum vitae, une synthèse de travaux de recherche réalisés depuis 2007 et le projet des recherches à venir.

Les travaux de recherche fondamentale ont été réalisés durant le master II et le doctorat, au sein des unités Inserm U676 et Avenir R05230HS. Les travaux de recherche clinique et de traitement d'image et de signal ont été réalisés au sein du pôle femme-parents-enfant du CHU de Reims, en collaboration croissante avec le laboratoire Centre de Recherche en Sciences et Technologies de l'Information et la Communication (CReSTIC, EA 3804) de l'Université Reims Champagne Ardenne (URCA).

Ce projet de recherche est intégré à notre dynamique de recherche locale : le projet hospitalo-universitaire « Cognition sociale et maladies neurodéveloppementales ».

1 CURRICULUM VITAE

1.1 ETAT CIVIL

Gauthier Loron, 43 ans (16 juillet 1979), marié, 2 enfants.

MCU-PH en physiologie médicale, spécialité hospitalière pédiatrie.

Université Reims Champagne-Ardenne, UFR Médecine - 51 rue Cognacq Jay, 51100 Reims

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1.2 PRESENTATION

J'ai effectué la majeure partie de mon cursus médical à la faculté de médecine de Reims, à laquelle je suis attaché. Durant mon internat, je me suis spécialisé en réanimation et en neurologie pédiatriques, en effectuant des stages en réanimation adulte, réanimation pédiatrique et laboratoire d'électrophysiologie. J'ai complété ce diplôme d'études spécialisées de pédiatrie par un diplôme inter-universitaire de neurologie pédiatrique et un diplôme d'études spécialisées complémentaires de réanimation néonatale. Cette orientation s'est concrétisée par la réalisation d'un clinicat dans le service de médecine néonatale et réanimation pédiatrique.

Deux années de disponibilité durant l'internat m'ont permis de réaliser un Master II en biologie de la reproduction (Unité Inserm U676 du Dr Gressens), puis une première année de thèse (Unité Avenir R05230HS/UMR 676 du Pr Olivier Baud, école doctorale Génétique, Cellule, Immunologie, Infectiologie, Développement, Paris V Cochin). J'ai poursuivi ce travail de thèse en parallèle de mon internat puis de mon clinicat.

Deux années de mobilité ont suivi ce clinicat, dans le service de réanimation pédiatrique du centre hospitalier universitaire de Sainte Justine, Montréal, Canada (2011-2013). Je m'y suis perfectionné en réanimation pédiatrique, j'y ai acquis des bases en simulation en santé, participé à un programme de recherche en soins respiratoires et introduit dans ce service le monitoring cérébral continu par EEG d'amplitude.

De retour à Reims, j'ai poursuivi mon activité de réanimation néonatale et pédiatrique en orientant ma pratique vers les soins intensifs neurologiques et le neurodéveloppement. J'ai participé à la création du service de soins critiques pédiatriques, dont je suis responsable de structure interne pour la partie médicale.

Soutenu par le Professeur de Physiologie Pierre MAURAN, qui connaissait mon souhait de développer enseignement et recherche, j'ai souhaité dès la fin de ma formation initiale m'orienter vers un parcours universitaire en Physiologie Médicale.

Depuis, j'anime les enseignements de physiologie à l'institut de formation aux soins infirmiers du CHU de Reims, de neurophysiologie aux étudiants LAS2, en médecine (FGSM3) et en école de manipulateurs d'électroradiologie médicale. Je participe au développement de nouvelles techniques pédagogiques dans notre faculté de médecine, notamment en y dirigeant la commission simulation.

Nous avons construit un projet de recherche s'intéressant à l'exploration fonctionnelle et morphologique du cerveau en développement, à l'étude de ses pathologies comme la prématurité et l'anoxo-ischémie.

Cet engagement hospitalo-universitaire s'est concrétisé en 2020 avec ma nomination en tant que MCU-PH en physiologie – spécialité hospitalière pédiatrique, auprès de la section 44-02 du CNU (physiologie médicale). En mars 2021, j'ai obtenu mon rattachement recherche au laboratoire CReSTIC.

1.3 CURSUS UNIVERSITAIRE

- 2007-2012 **Thèse de sciences**, spécialité **Neurosciences**.
École doctorale Génétique, Cellule, Immunologie, Infectiologie, Développement, Paris V Cochin. Équipe Avenir Inserm R05230HS du Pr Olivier Baud (UMR 676 – Hôpital Robert Debré, Université Paris VII Diderot).
« Effets systémiques et cérébraux du monoxyde d'azote inhalé chez le raton »
- 2009-2012 **Diplôme d'Études Spécialisées Complémentaires de réanimation néonatale**
Université de Reims
- 10/2009 **Diplôme d'Études Spécialisées de Pédiatrie**
Université de Reims
- 20/02/2008 **Thèse de doctorat en médecine**
« Electroencéphalogramme d'amplitude chez le prématuré. Étude des tracés de la première semaine de vie et corrélation au devenir. »
- 2006 -2007 **Master II Recherche**. Mention Biologie Cellulaire, Physiologie et Pathologie, spécialité Reproduction & Développement (Pr René Habert, Paris VII).
Unité Inserm 676, Dr Pierre Gressens, Hôpital Robert Debré, Université Paris VII Diderot. « Modifications d'expression et d'activité des métalloprotéines 2 et 9 dans un modèle d'excitotoxicité in vitro sur des cultures primaires neuronales, astrocytaires et microgliales murines. Interactions avec le BDNF. »
- 1998 - 2000 **Maîtrise de Sciences Biologiques et Médicales (M1)**.
Certificat « Biochimie et Biologie Moléculaire » et « Biotechnologie et Ingénierie Biomédicale » - Université de Médecine de Reims.
- 1997 – 2009 **Études médicales**.
UFR de médecine de Reims.

1.4 DIPLOMES UNIVERSITAIRES ET AUTRES FORMATIONS

- 2020 - 2021 **DIU de pédagogie en sciences de la santé**, Université Picardie Jules Verne, Amiens.
- 2018 **Formation simulation au debriefing difficile**, CUESIM de Nancy, février 2018.
- 2016 **Instructeur EPLS/RANP** (European Paediatric Life Support) de l'European Resuscitation Council (*détails au chapitre enseignement*).
- 2015 **Formation Generic Instructor Course (GIC)** de l'European Resuscitation Council.
- 2015 **Formation EPLS « European Pediatric Life Support »**.

- 2012 **Formation PALS « Pediatric Advanced Life Support »** Formation de réanimation pédiatrique effectuée en mobilité à l'université de Montréal, Canada.
- 2012 **Formation ATLS « Advanced Trauma Life Support »** Formation de prise en charge en urgence du polytraumatisé de l'American College of Surgeons. Université de Montréal, Canada.
- 2012 **Formation ECMO pédiatrique.** Cours siglé SIN8104 de l'Université de Montréal, Canada.
- 2005 - 2007 **D.I.U. de neurologie pédiatrique** - Université d'Amiens.
- 2005 - 2006 **CESAM** : Diplôme du centre d'enseignement de la statistique appliquée à la médecine. Université de Paris-Jussieu.
- 2004 - 2003 **D.I.U. de pathologie infectieuse pédiatrique** - Université de Poitiers.
- 1997 Baccalauréat Série S spécialité mathématiques.

1.5 TITRES HOSPITALIERS

- 2020 **Maître de conférence des universités – Praticien Hospitalier.** Spécialité physiologie médicale (CNU 44.02), spécialisation hospitalière : pédiatrie
- 2013-2020 **Praticien Hospitalier** dans le service de médecine néonatale et réanimation pédiatrique, CHU de Reims, Pr Bednarek, de novembre 2013 à septembre 2020. Concours de praticien hospitalier, épreuve de type I, spécialité pédiatrie, février 2015.
- 2011-2013 **Fellow à l'Université de Montréal et CHU Sainte Justine.**
- 2009-2011 **Chef de Clinique Assistant** dans le service de médecine néonatale et réanimation pédiatrique du Pr Morville, CHU de Reims.
- 2003-2009 **Interne des hôpitaux** de novembre 2003 à novembre 2009.

1.6 MOBILITES

- 2011-2013 **Fellowship à l'Université de Montréal et CHU Sainte Justine.**
A l'issue du clinicat réalisé dans le service de médecine néonatale et réanimation pédiatrique du CHU de Reims, j'ai suivi durant deux années le programme de fellowship du service de soins intensifs du CHU Sainte Justine, à Montréal, Qc, Canada. Ce service est composé de 20 lits de réanimation pédiatrique (médicale et post-chirurgie cardiaque). J'y ai exercé la fonction de moniteur clinique (Fellow). En parallèle j'ai participé à un travail de recherche en soins intensifs respiratoires sous la direction du Pr Juvet et contribué à implanter dans le service le monitoring par électroencéphalogramme d'amplitude. J'ai enfin suivi plusieurs enseignements en réanimation pédiatrique et ECMO, et participé à de l'enseignement par simulation, comme apprenant et enseignant.
- 2007-2008 Première année de thèse de sciences dans le laboratoire du Pr Olivier Baud (Équipe Avenir Inserm R05230HS /UMR 676 – Hôpital Robert Debré, Université Paris VII Diderot).
- 2006-2007 Master II recherche dans l'unité U676 du Dr Pierre Gressens.

1.7 INSCRIPTION A DES SOCIETES SAVANTES

- Depuis 2018 European Society of Paediatric and Neonatal Intensive Care : ESPNIC. Membre de la commission "NeuroCritical Care".
- Depuis 2017 Société française de physiologie.
- Depuis 2017 Société de réanimation de langue française.
- 2016 - 2019 Conseil scientifique du congrès des journées francophones de recherche en néonatalogie – Société française de néonatalogie. Mandat de 4 ans.

1.8 RESPONSABILITES LOCALES, REGIONALES, NATIONALES

- Depuis 2020 Responsabilité de la structure de réanimation pédiatrique et soins continus médicaux, Hôpital Américain, CHU de Reims.
- Depuis 2018 Responsabilité de la commission simulation, faculté de médecine de Reims.
- Depuis 2020 Co-responsable pédagogique DFASM2.
- 2016-2018 Membre du Conseil scientifique du réseau périnatal Champagne-Ardenne.
- Depuis 2016 Instructeur de la formation EPLS/RANP de l'European Resuscitation Council.
- Depuis 2015 Membre du conseil technique de l'école de puériculture (IRF CHU Reims).

1.9 REVUE D'ARTICLES

Participation au processus de revue pour les publications suivantes :

- Archives françaises de pédiatrie
- Gene
- Pediatric Critical Care Medicine
- PLOS One
- Pediatrics
- Children (Basel) MDPI
- Frontiers in Pediatrics
- European Journal of Pediatrics

1.10 PRIX, BOURSES, FINANCEMENTS

- 2020 Financement de thèse d'université obtenu pour Mme Cassandre Guittard, Comité Américain, 135000 euros sur 3 ans.
- 2021 AOL PrédANPa, Investigateur associé, environ 14000 euros.
- 2018 Financement du PHRCi « CALIN », 298000 euros.
- 2011 Bourse de jumelage de la fondation Sainte Justine pour réalisation d'un fellowship, 80000 dollars CAD.
- 2011 Bourse de Fellowship du Comité Américain, 60000 CAD (AMH, Reims)
- 2008 Session des primés des XIVe Journées Francophones de Recherche en néonatalogie, Paris.
- 2007 Prix de la Journée de Recherche Clinique du CHU de Reims
- 2006 Bourse de Master II du Comité Américain, 18000 €.

2 RESUME DU PROJET HOSPITALIER

Le pôle Femme-Parents-Enfant regroupe les services et consultations de pédiatrie générale et spécialisée, les urgences, la réanimation, la chirurgie pédiatrique, la pédopsychiatrie et le service de gynécologie-obstétrique. J'exerce mon activité clinique principalement en réanimation pédiatrique, dans les services de médecine néonatale, en consultation, et au laboratoire d'électrophysiologie pédiatrique.

2.1 SERVICE DE MEDECINE NEONATALE ET REANIMATION PEDIATRIQUE

Depuis janvier 2020, le service de réanimation polyvalente néonatale et pédiatrique a été transformé en :

- une unité de réanimation néonatale (18 lits de réanimation néonatale, 20 lits de soins intensifs),
- une unité de Réanimation pédiatrique (6 lits) et une unité médico-chirurgicale de soins continus (8 lits).

En tant que responsable de structure interne de l'unité de réanimation pédiatrique et de soins continus médicaux, j'y assure l'organisation des soins et la charge administrative. Nous comptons plus de 420 admissions annuelles en réanimation pédiatrique. Une équipe médicale de 5 équivalents temps-plein assure l'activité clinique de ce service et la continuité des soins. Je participe à l'activité clinique, organisée en semaines de service, à raison d'une semaine par mois, ainsi qu'aux gardes, 3 à 5 mensuelles.

Au-delà de ces activités cliniques, je suis présent quotidiennement au staff matinal de l'équipe et comme personne-ressource pour les situations de détresse neurologique. Dans ce cadre, j'interprète les monitorages cérébraux et les imageries cérébrales réalisées dans le service.

2.2 CONSULTATIONS

A raison de six vacances mensuelles, mon activité de consultation est ciblée sur :

- le suivi de l'enfant cérébrolésé,
- le suivi et dépistage de l'enfant né prématurément à risque neurodéveloppemental,
- la prise en charge des troubles du comportement et des troubles complexes des apprentissages de l'enfant né prématurément,
- la prise en charge des pathologies neurologiques de la première année de vie.

Je réalise en moyenne 450 consultations annuelles.

2.3 ÉLECTROPHYSIOLOGIE

Membre de l'équipe de neuropédiatrie, je participe à l'activité du centre d'explorations fonctionnelles neurologiques pédiatriques. J'y interprète les électroencéphalogrammes réalisés pour les enfants hospitalisés en néonatalogie, en réanimation pédiatrique et en consultation.

2.4 PROJET HOSPITALIER

Je souhaite renforcer et valoriser notre pratique de soins critiques pédiatriques. Ce projet passe par :

- Le renforcement de l'équipe médicale.
- L'accueil et la formation des médecins juniors et docteurs juniors, d'option réanimation pédiatrique, de médecine d'urgence, de pédiatrie, d'anesthésie réanimation.
- La coopération avec les spécialités interagissant fréquemment avec les soins critiques : hématologie/oncologie pédiatrique, chirurgie pédiatrique, urgences, services d'hospitalisation traditionnelle, au sein du CHU, du GHT et de la région.
- Le soutien au développement de la neurochirurgie pédiatrique au sein de notre CHU.
- La promotion d'activités de recherche médicale et paramédicale dans le service. Par exemple, un travail de recherche paramédicale sur l'apport de l'échographie dans la mise en place de voie veineuse est actuellement développé.
- La mise en place d'un curriculum de formation pour les étudiants de 3^e cycles concernant la reconnaissance et la prise en charge de l'enfant gravement malade.
- Le déploiement de l'approche par compétences pour les étudiants de 2^e cycle en stage dans l'unité.

3 ENSEIGNEMENT

3.1 SYNOPSIS

3 axes d'enseignement :

- Physiologie générale et neurophysiologie
- Nouvelles pédagogies et simulation
- Soins intensifs pédiatriques et néonataux

Score SIAPS de 551 points en mars 2022

3.2 FORMATION A L'ENSEIGNEMENT

En 2021 j'ai suivi l'enseignement du DIU de pédagogie active en sciences de la santé de l'université d'Amiens. Le mémoire, co-réalisé avec le Dr Sophie Deguelte et le Dr Maxime Hentzien, traitait du sujet suivant : « *Level of anxiety in medical student upon a first summative OSCE: Are OSCEs really that stressful for students?* »

J'ai également suivi une formation d'instructeur EPLS/RANP. La formation EPLS (European Paediatric Life Support) est un cours certifiant, proposé en formation initiale ou continue, coordonné et encadré par l'European Resuscitation Council. Similaire à des formations adultes de premier secours ou de réanimation (comme l'ALS), cette formation inculque les bases de la prise en charge en urgence d'un enfant gravement malade, sur deux jours de cours intensifs. La méthodologie associe auto-formation sur manuel, évaluation par QCM, cours en conférence, discussion ouverte et fermée, simulation. J'ai validé en 2015 la formation d'instructeur et je participe à ces formations en France.

3.3 RESPONSABILITE ADMINISTRATIVE D'ENSEIGNEMENT

Responsable de la commission simulation de la faculté de médecine de Reims.

Cette commission a été créée en 2018 et j'y coordonne les projets de simulation au sein de la faculté de médecine. Nous avons collectivement dressé un inventaire de formations proposées et des attentes au sein de notre faculté et notre CHU, établi un cahier des charges pour un centre de simulation, et mis sur pied une formation courte d'initiation à la simulation à destination des nouveaux entrants dans l'équipe d'enseignants de la faculté.

A présent nous développons un programme de patients standardisés et diffusons la pratique de la simulation en santé aux différents cycles des études médicales et paramédicales, ainsi qu'aux différents pôles hospitaliers.

Je suis également **Co-responsable pédagogie du DFASM2**. J'y seconde le Pr René Gabriel.

3.4 ENSEIGNEMENTS DE LA PHYSIOLOGIE

Depuis 2022 **Physiologie** LAS2 : 6 capsules vidéo avec autotest et 4 heures d'ED.

Depuis 2018 **Neurophysiologie** FGSM3 UEMED502 (10 à 12 heures annuelles).

Depuis 2017 **Cours de physiologie** en première année à l'Institut de Formation en Soins Infirmiers (10 heures/an).

Depuis 2017 Cours de **neurophysiologie** à l'Institut de Formation des Manipulateurs d'électroradiologie médicale, 5 heures annuelles.

3.5 ACTIVITES D'ENSEIGNEMENT HORS PHYSIOLOGIE.

3.5.1 DES/DESC, DIU, deuxième cycle des études médicales

Depuis 2017 Diplôme spécialisé d'études complémentaires de réanimation néonatale. Quatre heures par an. (Débit sanguin cérébral chez le prématuré et moyens d'étude, De la structure à la fonction en néonatalogie, Plasticité cérébrale et intervention précoce, Electroencéphalographie chez le nouveau-né, Annonce d'une mauvaise nouvelle – 8 heures par an)

Depuis 2017 DES de médecine d'urgence : urgences pédiatriques (Coma, particularités pédiatriques de l'arrêt cardiaque – 2 heures / an). Inter-région Est, Reims.

2017 et 2019 Particularités pharmacologiques de l'enfant : Séminaire Diplôme d'études spécialisées de pharmacie (2 heures). Inter-région Est, Reims, 23 janvier 2017 et 15 mars 2019.

Depuis 2016 Cours de neurologie pédiatrique au DIU de soins infirmiers pédiatriques, CHU de Reims : 4 à 6 heures / an.

3.5.2 Enseignement par simulation

Depuis 2018 Attestation « formation à la simulation » à destination des CCA-AHU. 15 heures / an.

Depuis 2017 Simulation « annonce de mauvaise nouvelle » auprès des internes DES de pédiatrie et médecins juniors de chirurgie. Huit à 12 heures / an.

Depuis 2015 Instructeur aux formations EPLS/RANP (Réanimation avancée néonatale et pédiatrique ou European Pediatric Life Support)

Depuis 2016 Institut régional de formation – Institut de puériculture. Enseignement aux gestes d'urgence par la simulation. Huit heures / an.

3.6 ENSEIGNEMENTS ANTERIEURS

2005-2020 Enseignement aux élèves sage-femmes de 1ère, 3e et 4e année. Huit à 10 heures de cours par an ciblant le développement cérébral et les pathologies neurologiques néonatales.

2013-2020 Formation Réanimation salle de naissance par la simulation in situ. Réseau périnatal Champagne-Ardenne.

2018 Enseignement post-universitaire auprès des médecins généralistes (réseau Épernay). Repérage et prise en charge des séquelles neurologiques de la grande prématurité.

2018 Cours au DES de pédiatrie Socle 1 (2 heures).

2009 - 2018 Cours magistraux deuxième cycle des études médicales UE2 - 8 – 11 (neuropédiatrie et réanimation pédiatrique).

2014-2017 Enseignement à l'école d'infirmiers anesthésistes D.E. (3 heures).

2007-2017	Formations au monitoring cérébral continu par EEG d'amplitude en réanimation néonatale. Mai 2007 CHU Reims - Juin 2007 Vélizy - Juin 2007 CHU Bordeaux - Avril 2009 CHU Caen - Septembre 2009 Cayenne - Septembre 2013 CHU Sainte-Justine, Montréal, Canada - Mars 2015 CHU Louis Mourier, Colombes - 2016 Liège - 2016 Nantes - 2017 Lyon HFME.
2009-2011	Participation à l'enseignement des étudiants en soins infirmiers.
2011 - 2013	Activité d'enseignement au CHU Sainte-Justine, Montréal, Canada : aux externes, moniteurs cliniques et résidents. Participation aux activités du centre de Simulation haute-fidélité du CHU Sainte-Justine.
2009-2011	Clinicat, participation à la formation des étudiants en médecine (sémiologie, pédiatrie), cours magistraux et au lit du malade.

3.7 PROJET D'ENSEIGNEMENT

3.7.1 Promouvoir la physiologie médicale à l'université de Reims

Je seconde le Pr Pierre Mauran dans l'enseignement de la physiologie à la Faculté de Médecine de Reims. Progressivement je reprends la responsabilité d'enseignements.

Je participe à l'harmonisation du programme et des objectifs d'apprentissage de physiologie pour les différents instituts de formation en soins infirmiers de notre région.

A long terme, mon objectif est de fédérer une équipe d'enseignants en physiologie médicale au sein de la faculté de Médecine de Reims.

3.7.2 Évoluer avec les nouvelles techniques pédagogiques

Les cours magistraux restent un moyen unique de présenter simultanément à un grand nombre d'étudiants les concepts de physiologie. Néanmoins d'autres approches peuvent s'ajouter, soit pour faciliter la compréhension de chaque étudiant, soit pour faciliter le transfert des connaissances. En somme, le défi est de combiner différents supports d'enseignement pour espérer toucher chaque étudiant et viser une appropriation durable des concepts fondamentaux.

Les pistes sont nombreuses, les nouveaux supports, quoique « modernes », n'ont pas tous démontré leur efficacité et sont souvent coûteux, en temps d'enseignant comme en ressources techniques ou financières. Conscients de ces nouveaux enjeux et écueils potentiels et nous suivons les pistes suivantes :

- Développer l'enseignement à distance (e-learning). La mise à disposition de supports de cours en ligne est une volonté forte de notre Doyen. Depuis la rentrée 2020, le nombre d'heures de cours en amphithéâtre a diminué pour toutes les disciplines. J'ai développé un nouveau curriculum de formation en ligne et des enseignements dirigés. Ces derniers sont animés sous forme de travail de groupe en amphithéâtre, suite à l'expérience acquise en DIU de pédagogie. Nous avons recours à un tutorat, relayé par des étudiants de la promotion supérieure, pour répondre aux questions des étudiants sur le forum des cours en ligne. C'est une occasion de toucher des étudiants qui se présentent moins en cours, puis d'éprouver la compréhension des concepts au cours de sessions plus interactives en amphithéâtre.

- L'utilisation de la simulation dans l'apprentissage de la physiologie médicale. Depuis la découverte de cette forme d'enseignement lors de ma mobilité montréalaise, je suis convaincu de l'intérêt de l'apprentissage par la simulation pour faciliter le transfert des connaissances, ainsi que cela a été démontré. En un sens, les séances de travaux pratiques offertes de longue date aux étudiants en physiologie ne sont pas très loin de certaines techniques de simulation. Mais l'évolution des moyens techniques (mannequins haute-fidélité) et des logiciels offrent de nouvelles possibilités que nous prospectons avec le Pr Pierre Mauran. Nous projetons un atelier basé sur un mannequin enfant haute-fidélité (Sim Junior) abordant la régulation du système cardiovasculaire. Nous évaluerons l'impact pédagogique de ces ateliers.

Ces initiatives locales seront modulées et orientées en fonction des travaux menés par les instances nationales, en particulier le Collège Français des Enseignants Universitaires de Physiologie en Santé.

4 PARCOURS EN RECHERCHE DE 2007 A 2022

4.1 ACTIVITE DE RECHERCHE PREDOCTORALE

L'internat fut l'occasion de découvrir la recherche clinique, sur le sujet de l'électrophysiologie en néonatalogie. J'ai réalisé pour la thèse de doctorat en médecine un atlas d'électroencéphalogramme d'amplitude (aEEG) chez le prématuré.

Ce traitement de signal « aEEG » occupant toujours une part importante de nos travaux de recherche, je propose d'en détailler ici le principe (Figure 1).

Maynard et Prior, deux électrophysiologistes anglais, l'ont conçu à la fin des années 60 dans l'ambition d'analyser simplement la récupération neurologique d'adultes au décours d'un arrêt cardiaque (Maynard, Prior, and Scott 1969). Associant filtrage progressif et compression temporelle, ce traitement met en exergue la variation – le delta – d'amplitude des ondes électroencéphalographiques privilégiant la bande de fréquence de 2 à 15-20 Hz. Ce delta est restitué sous la forme d'une bande d'amplitude selon une échelle semi-logarithmique, en fonction du temps (3,5 heures par largeur d'écran en général). Ne nécessitant que peu

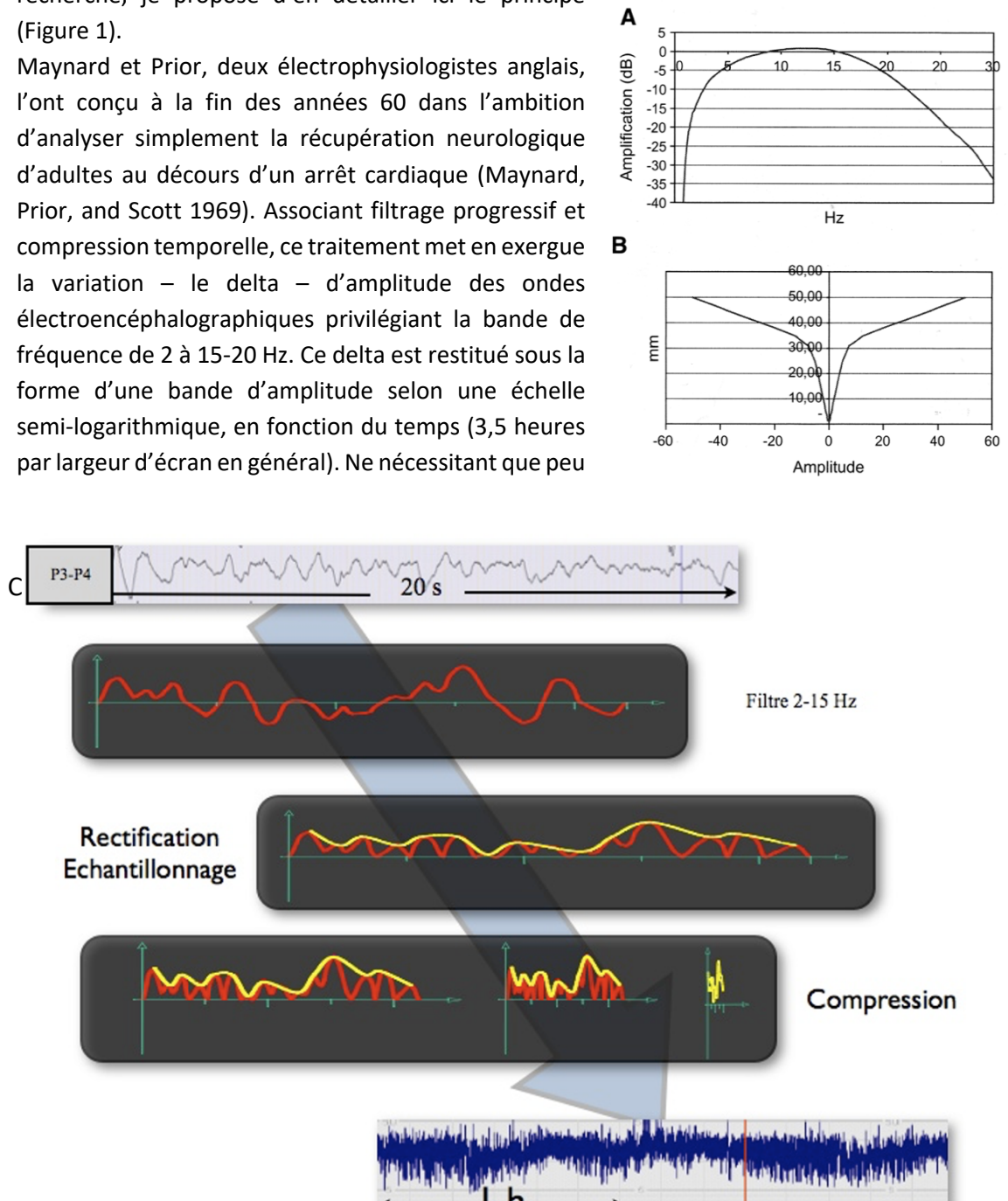


Figure 1 : Traitement de signal aEEG.

A : Filtre utilisé. **B :** Compression semi-logarithmique de l'amplitude. **C :** Etapes de traitement du signal

d'électrodes, sa mise en œuvre et sa lecture sont simples et rapides.

Resté confidentiel chez l'adulte, l'usage de l'aEEG pointe en néonatalogie durant la décennie 1985-1995, sous l'impulsion d'équipes d'Europe du Nord (Greisen à Copenhague, De Vries et Toet à Utrecht, Hellström-Westas et Rosen à Lund) (Toet, van Rooij, and de Vries 2008; de Vries and Toet 2006).

La conception de ce traitement de signal facilite la distinction des tracés très déprimés, discontinus et continus : ceci est pertinent en néonatalogie, par exemple dans l'encéphalopathie anoxo-ischémique (Bednarek et al. 2008). La capacité de ce traitement de signal à dégrossir le pronostic neurologique est très bonne (Toet, van Rooij, and de Vries 2008).

À la même époque, l'hypothermie thérapeutique s'imposait dans la prise en charge des nouveau-nés à terme souffrant d'encéphalopathie anoxo-ischémique. De nombreuses équipes et plusieurs recommandations nationales ont inclus les caractéristiques du tracé aEEG dans les critères d'initiation de l'hypothermie, en complément ou en alternative au gold standard EEG (Saliba and Debillon 2010; Biban et al. 2011).

Chez le prématuré, l'utilisation de l'aEEG était - et reste - plus délicate. Ce traitement de signal n'offre pas une résolution spatiale et temporelle suffisante pour détailler le contenu des bouffées d'activité, la synchronie, l'apparition et disparition successive de figures physiologiques, les figures pathologiques... Ces marqueurs importants de la maturation de l'électrogénèse sont l'apanage de l'EEG standard. Restent la proportion de tracé continu et discontinu, les variations du delta d'amplitude sur de longues périodes, que l'aEEG permet de facilement repérer. Au milieu des années 2000, les cliniciens manquaient de normes pour lire les tracés aEEG des prématurés et avancer dans l'intérêt clinique de ce monitoring.

Pour créer notre atlas chez le prématuré, nous avons recueilli les enregistrements aEEG durant la première semaine de vie de 128 enfants nés entre 23 et 36 semaines entre le 01/06/2004 et le 31/12/2005 (Figure 2). Nous avons documenté une modulation du tracé, y compris chez les enfants les plus immatures. Avec l'augmentation de l'âge gestationnel, l'amplitude minimum (la « marge inférieure » du tracé) augmente progressivement, tandis que l'évolution de l'amplitude maximale est biphasique, augmentant progressivement de 10 à 25 μV entre 23 et 28 SA, avant de redescendre progressivement, sous les 20 μV à 33 SA.

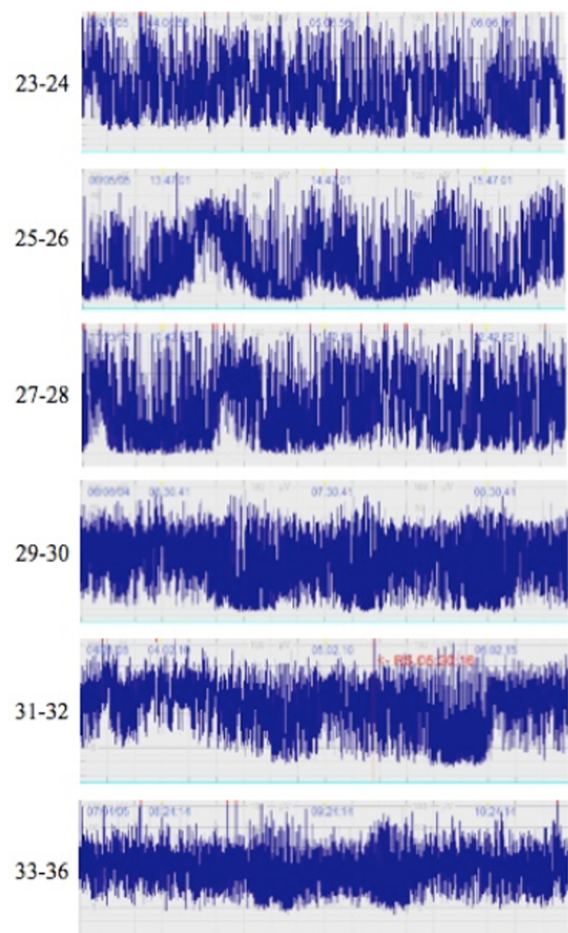


Figure 2 : Atlas aEEG chez le prématuré
Tracés type des premiers jours pour chaque âge

Ce travail de thèse a fait l'objet de plusieurs communications orales.

4.2 MASTER II ET DOCTORAT

La réalisation d'un Master II recherche puis d'une thèse d'université m'ont offert d'étudier le cerveau en développement sous un angle fondamental, utilisant des modèles de rongeurs.

4.2.1 Master II

Mon Master II recherche a été réalisé sous la direction du Dr Pierre Gressens, directeur de recherche de l'unité UMR 676. J'y ai appris la culture primaire de neurones immatures et de microglie. En collaboration avec le Dr Roselyne Garnotel du CHU de Reims et le Pr Nathalie Bednarek, nous avons étudié l'expression des métalloprotéinases MMP-2 et MMP-9. A partir de cultures cellulaires primaires (neuronale, astrocytaire, microgliale), nous avons révélé qu'un stress excitotoxique modifie l'expression de ces métalloprotéinases, différemment selon le type cellulaire observé (Bednarek et al. 2009; 2012).

La maîtrise de ces cultures cellulaires m'a permis plusieurs collaborations et publications avec le Pr Angela Kaindl et le Dr Romain Fontaine (Fontaine et al. 2008; Kaindl et al. 2012).

4.2.2 Thèse d'université : étude des effets cérébraux du monoxyde d'azote inhalé chez le raton nouveau-né

A l'issue de cette année de Master, j'ai débuté sous la direction du Pr Olivier Baud une thèse de sciences. Débutée lors d'une année de disponibilité, j'ai poursuivi la réalisation de cette thèse pendant la fin de l'internat et le clinicat.

Nous avons étudié, dans un modèle de raton nouveau-né comparable à la maturité du cerveau du prématuré humain, les effets de l'inhalation du monoxyde d'azote.

L'usage de ce gaz est courant en néonatalogie en traitement de l'hypertension artérielle pulmonaire néonatale. En tant qu'espèce hautement réactive, on ne prêtait au NO inhalé qu'une demi-vie extrêmement courte dans le plasma. Dès lors, l'inhalation de ce gaz n'aurait induit que des effets locaux. Pourtant, les travaux des équipes de Stamler, Gladwin, et d'autres, ont démontré l'existence d'une liaison réversible du NO aux nitrosothiols plasmatiques et à l'hémoglobine : son transport à distance était possible (Kim-Shapiro, Schechter, and Gladwin 2006; DeMartino et al. 2019; Seth et al. 2018).

Par ailleurs le NO endogène cumule de nombreux rôles dans la physiologie cérébrale : second messenger de la voie VEGF, stabilisateur synaptique, neurotransmetteur...

Avec ces démonstrations de transport réversible, connaissant les propriétés physiologiques importantes de la molécule, il devenait important d'évaluer l'impact de l'inhalation de NO sur le développement cérébral et le devenir des enfants prématurés (Baud et al. 2009; Mercier et al. 2009). Nous avons utilisé un modèle de raton nouveau-né exposé à des concentrations de iNO comparables à l'utilisation hospitalière de ce gaz.

Des portées et leurs mères étaient placées sous 5 à 20 ppm de NO de la naissance (P0) jusqu'au 7ème jour de vie postnatal (P7). Nous avons ensuite étudié l'histologie cérébrale, l'expression de gènes ciblés en qPCR, la concentration protéique en Western-blot, à P1, P7, P14, P21 (Figure 3).

L'angiogenèse cérébrale et la myélinisation étaient 7 jours plus précoces chez les animaux exposés au NO inhalé (iNO), que ce soit en densité cellulaire, concentration protéique ou

expression des mRNA. Les animaux contrôle atteignaient le même niveau de maturation à 21j au lieu de 14j de vie. L'exposition au NO était associée à une prolifération d'oligodendrocytes immatures et à une maturation anticipée des formes myélinisantes. Cette avance maturative n'avait pas d'incidence sur les fonctions cognitives à l'âge adulte, sur des tests comportementaux réalisés à P60 (Olivier et al. 2010).

Quel était le médiateur de cet effet pro-maturatif du iNO ? S'agissait-il d'un relargage à distance du NO inhalé ? Ou la mise en œuvre d'autres voies de signalisation ?

Le caractère hautement réactif du NO le rend très difficile à détecter et à mesurer. En utilisant une électrode ultrafine, nous avons détecté in vivo une augmentation de la concentration tissulaire de NO en comparaison à la concentration basale, à la suite de l'inhalation du NO.

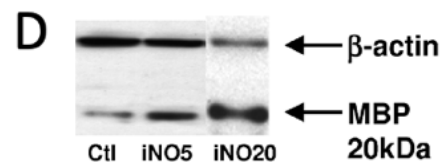
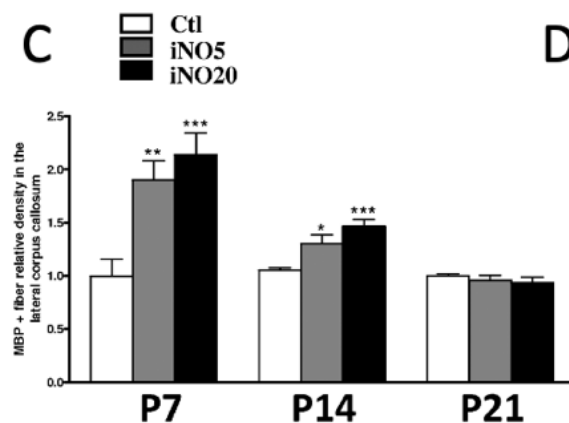
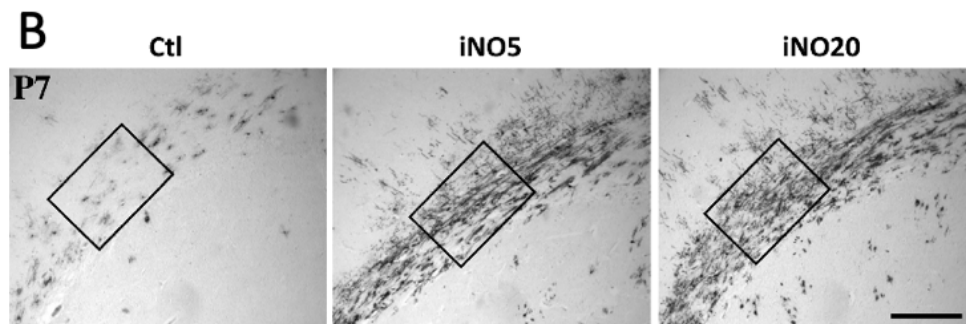
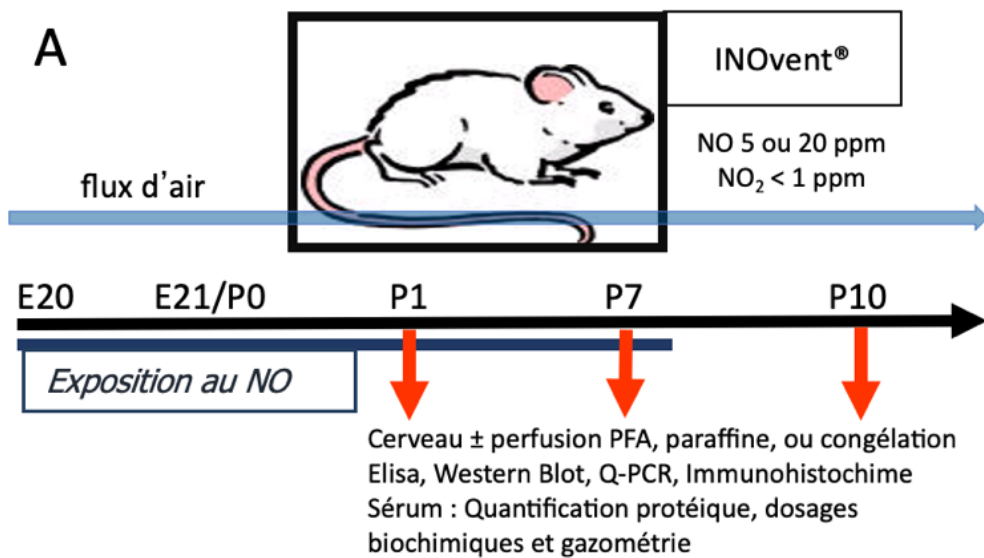


Figure 3 : Protocole iNO (A) et effets sur la myélinisation
B : Immunohistochime,
C : qPCR,
D : Western Blot

Les rôles du NO endogène et du couple VEGF/VEGFR2 ont été évalués via l'injection d'antagonistes : L-NAME pour inhiber les NO-synthases (e-, n- et i-NOS), SU-5416 comme antagoniste du récepteur principal du VEGF : VEGFR2. Dans les deux cas, l'inhalation de NO corrigeait les anomalies de myélinisation et d'angiogenèse induites par ces inhibiteurs.

Le NO endogène, produit par la NO-synthase dans la signalisation VEGF, a pour relais la NO-Guanylate cyclase et la production de cGMP. La concentration de cGMP dans le cerveau augmentait d'un facteur 5 à la suite de l'inhalation de NO. La thrombospondine est un antagoniste naturel de la signalisation NO-cGMP. L'injection de ABT-510, agoniste de TSP-1, abolissait les effets du iNO, confirmant l'hypothèse que les effets à distance reposent sur la signalisation NO-Guanylate Cyclase soluble-cGMP (Figure 4).

Nous avons soumis des rats à une agression excitotoxique par injection intracérébrale d'agonistes du glutamate. A P10, les rats exposés au iNO avant l'injection présentaient des lésions moins importantes, ainsi qu'une diminution de densité des microglies activées et des astrocytes. Cet effet neuroprotecteur était associé à une régulation de sous-unités des récepteurs au glutamate dès P5. Cet effet transcriptionnel semblait lié à la modulation de la signalisation pCREB/Akt. Plusieurs facteurs de transcription étaient ensuite régulés : PDGFR- α , Sema3F, les sous-unités des récepteurs au glutamate, et là encore, la Thrombospondine-1 (Pansiot et al. 2010).

Au total, nous avons démontré que le iNO est transporté de manière réversible et délivré au cerveau en développement. Il y exerce un effet pro-angiogénique et pro-myélinisant, via une signalisation cGMP, régulée par la thrombospondine-1. Plus encore, l'exposition prophylactique au iNO diminue l'impact d'une agression excitotoxique.

Ce travail de thèse a été soutenu en 2012. Les articles s'y rapportant sont disponibles en annexe.

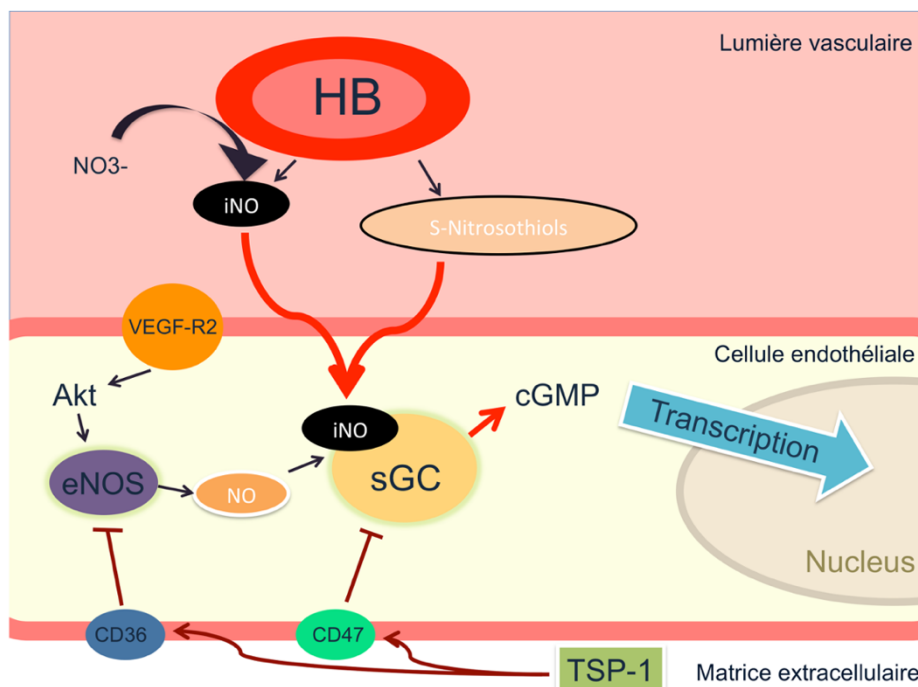


Figure 4 : Voie de signalisation du NO exogène (inhale)

le NO préalablement inhalé est libéré de son transporteur (NO₃⁻, S-nitrosothiol ou hémoglobine), diffuse dans le cytoplasme où il reproduit les effets du NO endogène. La thrombospondine-1 est un inhibiteur endogène de la voie de la eNOS-Guanylate cyclase.

4.2.3 Inflammation méningée et effet immun-modulateur d'une quinolone

Parallèlement, nous avons mis en évidence les effets anti-inflammatoires d'une quinolone dans un modèle d'inflammation méningée murin.

La substance blanche du prématuré - en particulier la lignée oligodendrocytaire - est très vulnérable à l'inflammation. Qu'elle soit systémique ou cérébrale, l'inflammation est un facteur de risque majeur de lésion cérébrale périnatale.

Nous avons utilisé un modèle de sepsis à E. Coli chez des rats à 5 jours de vie, modèle qui induit une inflammation méningée sans méningite. Comparé à l'antibiotique de référence cefotaxime, la ciprofloxacine diminuait l'activation microgliale et prévenait le retard de myélinisation induit par l'inflammation. Celle-ci entraînait une diminution d'expression des sémaphorines 3A et 3F, expression restaurée sous ciprofloxacine. Les effets immuno-modulateurs de la ciprofloxacine étaient confirmés dans un autre modèle de lésion cérébrale périnatale, non infectieux. Ce travail a fait l'objet d'une publication dans *Annals of Neurology*, disponible en annexe (Loron et al. 2011).

4.3 ACTIVITE DE RECHERCHE POST-DOCTORALE

Depuis le doctorat, j'ai orienté mon activité vers la recherche clinique, ciblée sur les investigations non invasives du cerveau en développement et certaines de ses pathologies. Ce projet est le fruit d'une collaboration avec le professeur Nathalie Bednarek, néonatalogiste et neurologue pédiatrique, le Professeur Nicolas Passat, directeur du laboratoire CRESTIC. Y participent également le Professeur Pierre Mauran, physiologiste et cardiologue pédiatrique et des membres de l'équipe médicale néonatale et pédiatrique. Au sein de cette thématique, je travaille plus sélectivement sur les moyens d'étude non-invasifs reposant sur l'imagerie cérébrale (échographie-doppler et IRM cérébrales) et sur l'électrophysiologie, standard et d'amplitude.

4.3.1 Travaux sur l'EEG d'amplitude

4.3.1.1 *Vers un atlas d'aEEG pédiatrique*

Nous avons abordé plus haut l'essor du monitoring aEEG en néonatalogie.

Chez l'enfant plus grand, utiliser cette approche pour suivre la récupération du tracé de fond après sa dépression reste pertinente. Les premiers travaux publiés montrent que la vitesse de récupération est pronostique. L'équipe de réanimation pédiatrique de Nantes a documenté la valeur pronostique de ce monitoring au décours de l'arrêt cardiaque en pédiatrie, travaux auxquels j'ai collaboré (Bourgoin, Barrault, Loron, et al. 2019; Bourgoin, Barrault, Joram, et al. 2019).

Un des obstacles à l'utilisation de ce monitoring chez l'enfant plus grand est l'absence de normes, sachant que l'activité électrique spontanée et son amplitude évoluent avec l'âge chez l'enfant et varient en fonction des stades de vigilance.

Nous avons, au cours du travail de thèse du Dr Margaux Descharmes et que j'ai encadré, travaillé sur des normes en partant de la technique de référence en électro-encéphalographie : l'EEG standard.

Nous avons sélectionné rétrospectivement des électroencéphalogrammes normaux à partir de la base de données du laboratoire d'électrophysiologie pédiatrique du CHU de Reims. Leur

normalité a été vérifiée par double lecture. La tendance « EEG d'amplitude » a été dérivée pour chaque dipôle via le logiciel Micromed®. Après exclusion des artéfacts, nous avons manuellement caractérisé les marges des tracés, jusqu'à six périodes de dix minutes pour chaque tracé aEEG, en veille et sommeil lent.

Quatre-vingt-huit tracés aEEG ont été dérivés, 4620 mesures réalisées. Un abaque des valeurs médianes et interquartiles des marges a été établi, de 1 mois à 18 ans, ainsi que des tracés types pour chaque dérivation (Figure 5). En état d'éveil, les marges inférieure et supérieure augmentent entre 1 mois et 6 ans de 10 μV à 15 μV et de 50 μV à 85 μV , respectivement. Elles tendent ensuite progressivement vers 75 μV et 10 μV à 18 ans. Les amplitudes en sommeil lent sont plus élevées, avec une grande variabilité inter-individuelle.

Nous disposons maintenant d'un atlas et de valeurs médianes avec quartiles en fonction de l'âge et du stade de vigilance. Ce travail a fait l'objet d'une communication orale au congrès européen de réanimation pédiatrique en 2019. Une équipe anglaise vient de publier la même méthodologie dans Archives of Disease in Childhood, nous espérons dans une réponse aux auteurs diffuser également nos résultats. Les principales figures et résultats du travail de thèse sont disponibles en annexe.

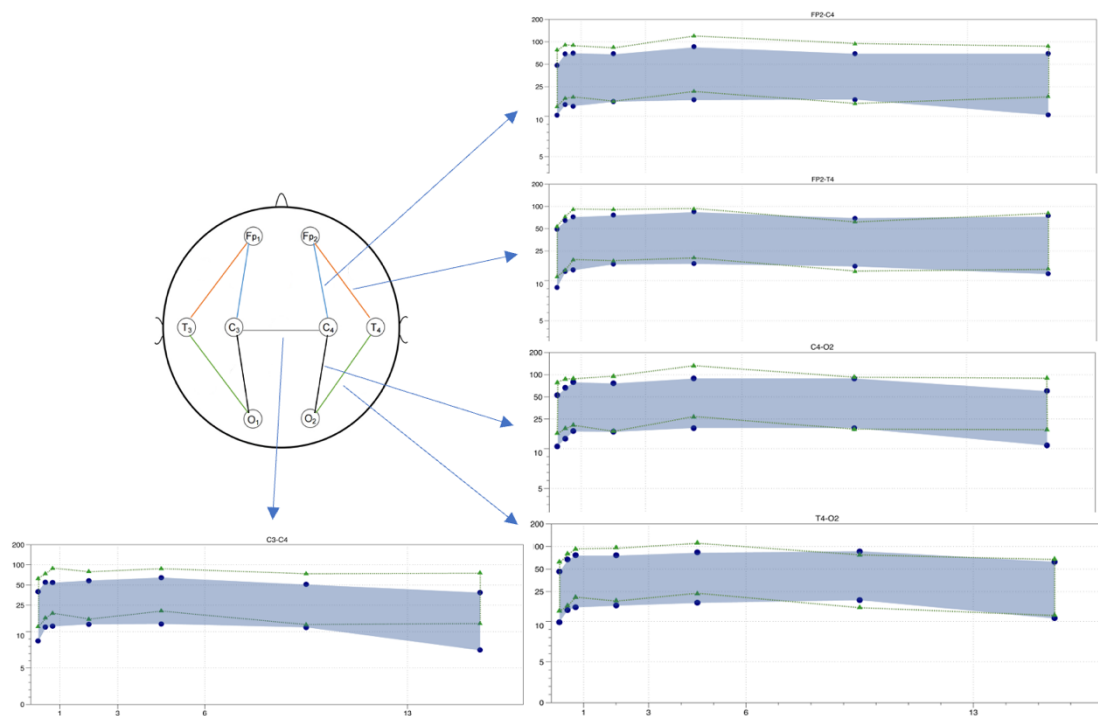


Figure 5 : Normes aEEG chez l'enfant.

Chaque graphe décrit les amplitudes (en μV) pour une dérivation selon la nomenclature 10-20

4.3.1.2 *Monitoring cérébral dans la méningite du nourrisson de moins d'un an.*

Il est chez le jeune enfant difficile d'apprécier le pronostic d'une méningite aiguë de façon précoce. De plus les convulsions - y compris infracliniques - semblent fréquentes dans cette population.

Nous avons évalué l'apport d'un monitoring aEEG chez l'enfant de moins de 1 an hospitalisé en réanimation pour méningite.

Il s'agissait d'une étude observationnelle, monocentrique, rétrospective sur dix ans. Vingt-cinq patients étaient inclus. Les enregistrements étaient initiés en moyenne dans les six heures suivant l'admission, pendant près de cent heures en moyenne et leur qualité était bonne, témoignant de la faisabilité de la technique. Dans cette population, 72% des patients ont présenté des crises comitiales cliniques et/ou infracliniques durant leur hospitalisation. Onze enfants ont présenté des crises infracliniques, dont deux exclusivement.

La survenue de crises comitiales, a fortiori de status epilepticus, et le score de tracé de fond étaient significativement associés à un pronostic défavorable (score « Pediatric Cérébral Performance Category ou PCPC ≥ 4 à un an).

Ce travail a fait l'objet d'un travail de mémoire de DES (Dr Jonathan Beck) et sa mise en forme pour publication a constitué le travail de thèse du Dr Cécile GROSJEAN, disponible en annexe (Beck, Grosjean, et al. 2022).

4.3.1.3 Monitoring cérébral dans le traumatisme crânien pédiatrique

Nous avons utilisé une approche similaire dans le traumatisme crânien pédiatrique. Sa prise en charge aiguë repose sur un monitoring multimodal pour limiter les lésions secondaires, mais la pertinence de l'électro-encéphalogramme dans cette indication n'a pas été étudiée.

Nous avons dans un travail rétrospectif effectué une première analyse de l'apport diagnostique et pronostique de la tendance « EEG d'amplitude » dans le traumatisme crânien grave.

Nous avons recueilli les données cliniques, électrophysiologiques et thérapeutiques, les imageries, le devenir des enfants hospitalisés en réanimation pédiatrique au CHU de Reims pour traumatisme crânien modéré ou sévère, entre le 01/01/2015 et le 31/08/2018, et ayant bénéficié d'un monitoring cérébral continu par EEG d'amplitude.

Nous avons caractérisé l'aEEG par période de 6 heures selon sa qualité, la classification d'Hellström-Westas, les valeurs moyennes des marges inférieure et supérieure, la présence de modulation, la présence de crises.

Les corrélations électro-cliniques ont été étayées par des tests de Student, Khi2 et ANOVA selon les variables.

Pendant la période considérée, 41 patients ont été hospitalisés avec un traumatisme crânien modéré ou sévère, dont 27 ont eu un aEEG. En moyenne, l'enregistrement débutait dans les 4 premières heures pour 93 heures. La présence d'une modulation avant 6h et 12h de vie était significativement associée à un meilleur devenir. L'existence de crises convulsives n'était pas péjorative dans cette observation.

Ce travail est en cours de rédaction pour publication. Il a fait auparavant l'objet de deux travaux que j'ai encadrés : une thèse de médecine (Dr Emilie Druot), avant d'être développé et enrichi par le Dr Perrine Venot sous forme de mémoire de DIU de neuro-réanimation. Il a été présenté sous forme de poster à la société française de neurophysiologie clinique.

A présent, une méthodologie prospective est nécessaire pour évaluer la pertinence pronostique de l'outil, voire pour tester si la présence d'un monitoring continu améliore la prise en charge et le devenir des patients.

4.3.2 Abaques de vélocité doppler chez le grand prématuré

La deuxième approche développée ces dernières années pour l'étude du cerveau en développement est l'étude de la perfusion artérielle, en utilisant la technologie de doppler dite « ultrarapide » (Ultrafast®). Cette technologie bénéficie des avancées informatiques pour exploiter une fréquence très élevée d'émission d'ultrasons (1000 Hz et plus), simultanément sur l'ensemble du plan (Demené et al. 2019).

En comparaison au doppler conventionnel, l'échographie par doppler Ultrafast® offre une meilleure résolution et permet d'analyser plusieurs spectres Doppler *a posteriori* sur n'importe quel vaisseau du plan considéré. Nous avons pu mesurer de façon inédite les vélocités de différentes artères intracérébrales chez le prématuré avec cette technique. Il nous est apparu pertinent de commencer par étudier les vélocités en situation de base chez les enfants prématurés, pour établir des abaques, avant de nous intéresser à des situations pathologiques.

Dans un travail prospectif, nous avons confirmé la fiabilité de la technique de doppler Ultrafast® par rapport à la méthode de référence par doppler pulsé dans l'exploration vélocimétrique des six artères intracérébrales principales. Nous avons

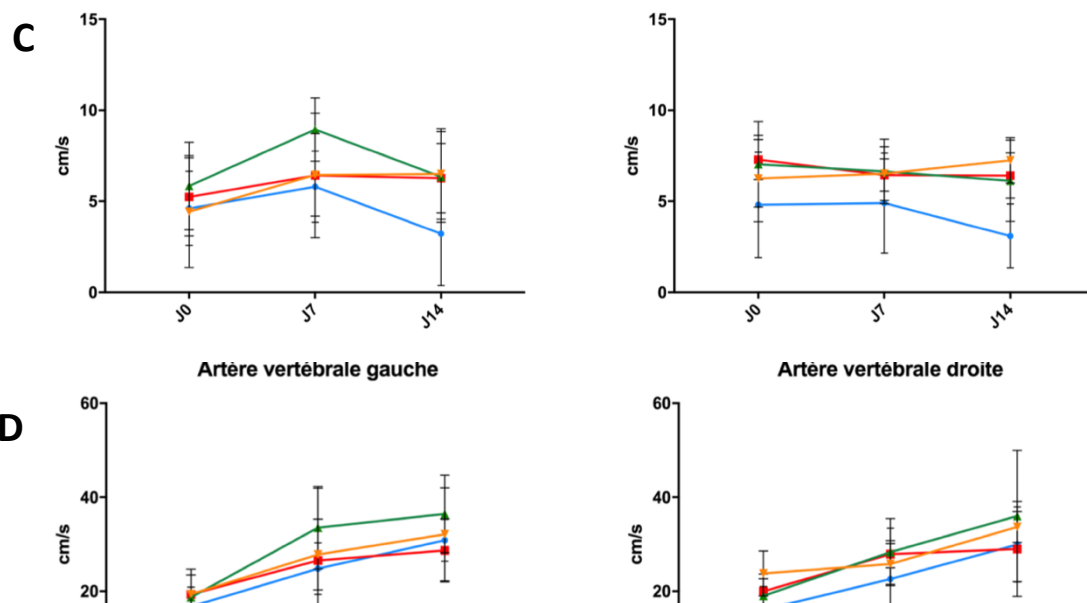
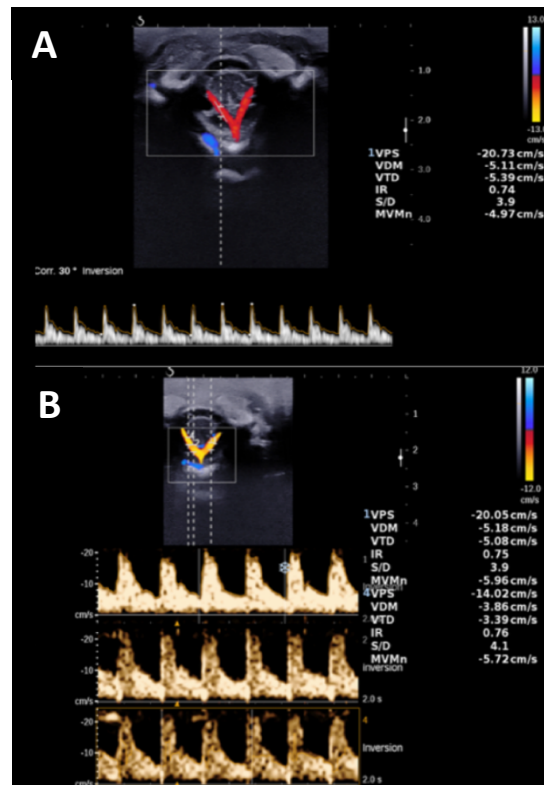


Figure 6 : Vélocités artérielles chez le prématuré.

A et B : Spectres doppler sur les artères vertébrales via le foramen ovale. C et D : Evolution des vélocités systoliques (C) et diastoliques (D) en cm/s sur ces mêmes artères, sur les 15 premiers jours de vie.

ensuite établi suivant l'âge gestationnel des enfants des normes de vitesse pour chaque artère sur les quatorze premiers jours de vie postnatale.

Cette étude incluait les enfants naissant avant 32 semaines d'aménorrhée. Une échographie par doppler pulsé puis par doppler Ultrafast® étaient réalisées à J0, J7 et J14 de vie avec exploration des vitesses télédiastoliques, télésystoliques et de l'index de résistance pour les artères cérébrales antérieure, moyenne, postérieure, du tronc basilaire et des artères vertébrales droite et gauche.

Vingt-et-un grands prématurés ont été inclus et 59 échographies cérébrales ont été effectuées. Les mesures des dopplers pulsés et Ultrafast® étaient bien corrélées. La médiane, les 10e et 90e percentiles étaient corrélés à l'âge gestationnel et au poids de naissance. L'évolution des vitesses durant les sept premiers jours montrait une augmentation des vitesses télésystoliques et télédiastoliques entre le premier et le septième jour de vie.

La figure 6 présente un extrait de ce travail, pour les artères vertébrales. Les figures pour l'ensemble des axes étudiées sont disponibles en annexe. Ce travail, co-encadré avec le Pr Nicolas Passat et le Pr Fabrice Wallois (Responsable du Master II, professeur de physiologie à l'Université Picardie Jules Verne) a été conduit par le Dr Hélène Meunier dans le cadre de son Master II Recherche.

Il semble intéressant de poursuivre ce travail en comparant ces vitesses artérielles cérébrales à l'hémodynamique systémique d'une part (présence d'un canal artériel ouvert, notamment) et à la micro-vascularisation et la fonction cérébrale (NIRS lente et rapide, électrophysiologie) d'autre part.

4.3.3 Analyse morphologique : échographie et IRM cérébrales

L'imagerie du cerveau en développement est un sujet crucial pour les enfants nés prématurément.

Chez le prématuré, les agressions inflammatoires, hypoxiques, ischémiques, sont souvent intriquées dans la genèse des lésions cérébrales. Ces dernières sont dépistées par l'imagerie traditionnelle : échographies transfontanellaires réalisées de façon séquentielle au chevet du patient, ou imagerie par résonance magnétique à terme corrigé dans les services où elle est proposée.

Les lésions ainsi identifiées peuvent être des hémorragies intra-ventriculaires, parenchymateuses, encéphalique ou cérébelleuse, ou des lésions de leucomalacie périventriculaire. Ces lésions dites « qualitatives » sont associées, en fonction de leur taille, de leur topographie, de leur caractère uni ou bilatéral, à certaines séquelles observées chez les grands prématurés : troubles moteurs, déficits cognitifs sévères, polyhandicap.

Au-delà de ces anomalies dites « qualitatives », des analyses morphologiques plus fines montrent la fréquence, chez les grands prématurés, d'altérations persistantes de croissance (volume, gyration) et de connectivité. Nous avons développé cette thématique dans un article de revue disponible en annexe (Vo Van, Alison, et al. 2022) Ces altérations semblent davantage reliées aux difficultés d'apprentissage, comportementales, psychologiques et aux troubles des fonctions exécutives retrouvés chez près de la moitié des enfants nés prématurément.

La mesure de volumes cérébraux régionaux n'est pour l'heure pas accessible en routine, même si de nombreuses équipes – dont la nôtre – travaillent sur ce sujet.

Plus accessible en pratique quotidienne, certaines mesures à l'IRM en deux dimensions semblent bien corrélées aux volumes régionaux (S. Nguyen The Tich et al. 2009; Sylvie Nguyen The Tich et al. 2011). Nous avons, dans le travail suivant, étudié la pertinence de l'échographie pour ces mesures.

4.3.3.1 Metrics ETF

Ce travail prospectif visait à comparer la précision de l'échographie et de l'IRM à mesurer en deux dimensions des structures cérébrales, chez des enfants nés prématurément, parvenus à terme corrigé.

L'IRM et l'échographie ont été réalisées à terme corrigé, chez des enfants nés avant 32 semaines d'aménorrhée. Les données démographiques et les résultats des échographies réalisées pendant l'hospitalisation ont été recueillis à partir des dossiers médicaux.

Les échographies ont été réalisées le même jour que l'IRM cérébrale. Deux néonatalogistes expérimentés répertoriaient les lésions qualitatives et réalisaient les mesures 2D IRM et échographiques en aveugle des données cliniques des patients. Ces mesures 2D comprenaient : les diamètres bifrontal, bipariétal osseux et parenchymateux, cérébelleux, la taille des espaces sous arachnoïdiens, la largeur des carrefours ventriculaires droits et gauche, la longueur du corps calleux et son épaisseur au genou antérieur, la hauteur et la profondeur du vermis et la profondeur du 4^e ventricule.

La corrélation des mesures 2D et les accords intra- et inter- observateurs ont été évalués à l'aide du coefficient de Pearson, des graphiques de Bland-Altman et du coefficient intra-classe.

Cent-deux enfants nés prématurément ont été inclus. Les performances d'un protocole « tout échographique » comparé à un protocole « ETF séquentielles et IRM à terme » étaient identiques pour la détection des hémorragies de haut grade et proches pour les hémorragies de bas grade. En revanche, seule l'IRM dépistait les lésions nodulaires de la substance blanche. Aucun enfant ne présentait d'infarctus cérébelleux à l'imagerie. Les accords intra- et inter-observateurs étaient excellents pour toutes les mesures 2D, sauf pour l'épaisseur du corps calleux et le diamètre antéro-postérieur du vermis. L'IRM et l'échographie ont montré une

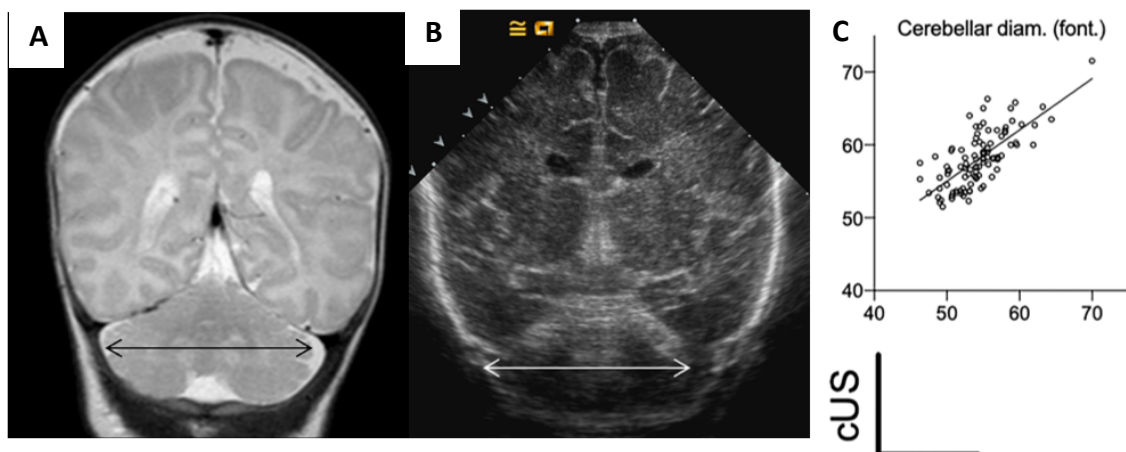


Figure 7 : Metrics ETF

Exemple de la corrélation de la mesure du diamètre transcérébelleux en IRM (A) vs ETF (B), et corrélation linéaire (C)

corrélation bonne à excellente pour les diamètres bipariétaux cérébral et osseux, la longueur du corps calleux, les diamètres trans-cérébelleux et des ventricules latéraux. La figure 7 présente un extrait de ces résultats : la corrélation de la mesure du diamètre trans-cérébelleux. L'ensemble des résultats figure en annexe dans l'article original. En revanche les mesures de l'épaisseur du corps calleux et des dimensions du vermis étaient faiblement corrélées. La précision de la mesure est probablement affectée soit par la petitesse de la structure, soit par la variabilité de l'angle d'incidence en échographie.

Ainsi, l'échographie cérébrale se révèle un outil fiable pour évaluer certaines mesures 2D du cerveau en développement. La répétition de ces mesures échographique pendant le séjour en néonatalogie permettrait de réaliser des courbes de croissance de plusieurs structures cérébrales. D'autres études permettront de déterminer si ces courbes sont des marqueurs pertinents du devenir neurologique.

4.3.3.2 *Segmentation automatisée de l'IRM cérébrale chez le nouveau-né*

Au sein du laboratoire du CRESTIC, nous participons à la mise au point d'un algorithme de segmentation automatique de l'IRM cérébrale chez le nouveau-né à terme. Y sont associés le Pr Nicolas Passat, le Pr Bednarek et Guillaume Dollé, ingénieur de recherche. Le doctorant Pierre Cettour-Jannet, Quentin Delennoy, ingénieur de recherche, et l'étudiante en Master Margaux Alloux ont également participé à son développement.

Comme décrit ci-dessus, comprendre les atteintes du cerveau en développement implique d'aller au-delà de la simple recherche de lésions sur l'IRM. Nous avons besoin d'outils de traitement d'image disponibles en routine.

Les quelques algorithmes déjà mis au point restent confidentiels, la validation des résultats obtenus et leur reproductibilité sur d'autres cohortes restent des points faibles. Ce projet était un des volets de l'ANR Maïa, reliant notamment l'équipe MIPA du laboratoire CRESTIC, le service de médecine néonatale du CHU de Reims, le laboratoire du Pr Fabrice Wallois à Amiens (GRAMFC), l'institut CNRS-IRISA, Kitware SAS et l'institut Mines-Telecom Bretagne (ANR-15-CE23-0009).

La méthode s'appuie sur l'utilisation de réseau neuronaux antagonistes. La première étape associe la reconstitution d'images super-résolues et la segmentation, la deuxième étape est la sélection d'images (Cettour-Janet et al. 2019).

Les IRM T1 3D issues d'EPIRMEX et la base de données DHCP ont permis l'entraînement de l'algorithme sur le supercalculateur de l'URCA.

Dans ce travail j'ai participé à l'encadrement du Dr Hélène Meunier au cours de son stage de Master II pour la réalisation des segmentations manuelles (établissement de vérité-terrain).

EPIRMEX

Ce projet de recherche national visait à corréler l'IRM cérébrale réalisée à terme au devenir cognitif des grands prématurés. Il s'agit d'une étude ancillaire du projet EPIPAGE-2, qui a inclus 5170 enfants nés avant 32 semaines entre le 28 mars 2011 et le 31 décembre 2011, recueilli les données démographiques, cliniques et de suivi jusqu'à 12 ans.

Dans le sous-groupe Epirmex, 581 enfants issus de 12 centres hospitaliers ont bénéficié d'une IRM cérébrale à terme – ce qui n'est pas une investigation de routine dans cette population. La relecture centralisée et experte de ces examens a rassemblé des néonatalogistes de Rouen, Tours, Marseille et Reims. Les fichiers DICOM des images étaient également centralisés pour traitement d'image.

J'ai également sélectionné les imageries cérébrales pour l'entraînement de l'algorithme. En effet les artefacts, la variabilité des paramètres d'acquisition, la présence de lésions, etc... pourraient fausser l'apprentissage de l'algorithme. Il a donc fallu passer en revue les IRM 3D T1 de la base de données Epirmex pour un « nettoyage » de la base d'entraînement.

4.3.4 Investigations morphologiques et électrophysiologiques dans l'encéphalopathie anoxo-ischémique

4.3.4.1 Description du PHRC Lytonepal

Le PHRC LyTONEPAL (Long Term Outcome of Neonatal EncePhALopathy) est une étude épidémiologique observationnelle prospective multicentrique française (Debillon et al. 2018). Ce PHRC national est porté par le Professeur Thierry Debillon, du CHU de Grenoble, et son volet ancillaire par le Pr Nathalie Bednarek. Soixante-huit unités de réanimation et soins intensifs néonataux (22 sur les 23 anté-régions françaises) ont participé. Entre septembre 2015 et mars 2017, ont été inclus les nouveau-nés d'âge gestationnel supérieur ou égal à 34 SA présentant une détresse neurologique précoce avec des signes cliniques d'encéphalopathie anoxo-ischémique modérée à sévère (grades II et III de la classification de Sarnat et Sarnat) ainsi que des critères biologiques d'asphyxie périnatale sur le pH au cordon ou dans la première heure de vie (acidose avec $\text{pH} < 7$ et base deficit > 16 mmol/L et/ou lactates > 11 mmol/L). L'objectif principal était de déterminer les paramètres périnataux, démographiques, cliniques et biologiques, prédictifs de décès ou de séquelles neurodéveloppementales modérées à sévères à trois ans.

Le volet principal de l'étude correspond à un recueil extensif des données obstétricales, périnatales (cliniques, paracliniques et biologiques) et des données de suivi, sur « Case Report Form » uniquement.

Un volet ancillaire, sur un échantillon des centres participants, ajoutait la collecte 1/ d'échantillons sanguins sans prélèvement supplémentaire (« fonds de tubes ») 2/ des fichiers DICOM des IRM et 3/ des données sources électrophysiologiques (EEG standard et d'amplitude).

4.3.4.2 Premiers résultats

Durant la période d'inclusion, 794 nouveau-nés furent inclus sur les 844 recensés. Les données du suivi à 3 ans sont en cours de consolidation pour l'exploitation complète de cette base de données. Le Dr Jonathan Beck, en cours de thèse de sciences, a notamment travaillé sur les IRM réalisées dans cette population. J'ai collaboré à la mise au point de deux publications du Dr Beck sur ce sujet (Beck et al. 2021; Beck, Loron, et al. 2022). Succinctement, ces travaux ont mis à jour le panorama de l'atteinte des structures cérébrales chez les patients présentant une encéphalopathie anoxo-ischémique à terme, sur un échantillon de grande taille (données IRM de 520 enfants). Si les atteintes des noyaux gris centraux sont très fréquentes, elles apparaissent rarement isolées. En outre, deux grands tableaux se dessinent : celui des atteintes prédominant sur les noyaux gris, et celui des atteintes prédominant sur la substance blanche. Nous formulons l'hypothèse que ces deux tableaux répondent à des cinétiques et chronologies d'anoxie différente, hypothèse en cours de test sur les travaux actuels et à venir.

Le projet ancillaire LyTONEPAL comprenait, outre les prélèvements biologiques, l'accès aux données sources des examens paracliniques. Nous avons organisé leur relecture approfondie et centralisée.

La relecture centralisée des IRM de 190 patients est en cours. Le panel d'expert associe Nathalie Bednarek (Reims), Marianne Alison (Robert Debré), Lucie Hertz-Pannier (NeuroSpin), Catherine Gire (Marseille), Philippe Vo Van (Lyon), Jonathan Beck (Reims).

Au sein de LyTONEPAL ancillaire, je suis particulièrement impliqué dans l'exploitation des données électrophysiologiques. Nous avons réuni deux panels d'experts pour la relecture centralisée des EEG et aEEG. Y ont collaboré 1/ pour le groupe EEG : Fabrice Wallois, Émilie Bourel-Ponchel, Laure Routhier (Amiens), Catherine Delanoë (Robert Debré), Marie-Dominique Lamblin (Lille), Catherine Gire (Marseille), Ines Damak (Troyes) Nathalie Bednarek, Margaux Descharmes et moi-même (Reims) ; 2/ pour le groupe aEEG : Antoine Giraud (Saint-Etienne), Philippe Vo Van (Lyon), Laura Menvielle, Jonathan Beck et moi-même (Reims). Ces relectures centralisées sont terminées, le nettoyage des bases de données est en cours ainsi que l'analyse exploratoire de ces données. Nous détaillerons les projets à venir dans la section « projet de recherche ».

ACTIVITES D'ENCADREMENT

4.4 THESE D'UNIVERSITE

2021 *En cours*. Co-direction avec le Pr Stéphanie Cailles (Laboratoire C2S) de Mme Cassandra Guittard, dans le cadre du projet CALIN

4.5 THESE D'EXERCICE POUR LE DOCTORAT DE MEDECINE

2022 (en cours) Madame Sirine Boussena. Publication « aEEG dans le traumatisme crânien grave et modéré de l'enfant ».

2022 Dr Cécile Grosjean. Publication de la valeur pronostique du monitoring aEEG dans les méningites aiguës avant 1 an de vie.

2017 Dr Margaux Descharmes. « Electroencéphalogramme d'amplitude chez l'enfant : tracés de référence ».

2014 Dr Emilie Druot. « Electroencéphalogramme d'amplitude chez le traumatisé crânien : faisabilité et bénéfices de la technique ».

4.6 ENCADREMENT DE MASTERE 2 RECHERCHE

2017 Dr Hélène Meunier. « Développement d'outils non invasifs de mesure de la perfusion cérébrale et de la croissance cérébrale chez le prématuré. Réalisation d'un atlas de segmentation IRM et de valeurs de référence de vitesses artérielles par échographie doppler ultrafast ».
M2 co-dirigé en collaboration avec le Pr Fabrice Wallois, Université Picardie-Jules Verne.

4.7 MEMOIRE DE DES DE PEDIATRIE

2018 Dr Hélène Meunier. « Vélométrie par doppler Ultrafast® de la vascularisation cérébrale des grands prématurés en période néonatale ».

2017 Dr Jonathan Beck « Electroencéphalogramme d'amplitude et méningite de la première année de vie ».

4.8 MEMOIRE DE DIU

2018 Dr Perrine Venot : « Valeur prédictive du monitoring par aEEG chez le traumatisé crânien pédiatrique ». Diplôme Universitaire de Neuroréanimation.

5 PUBLICATIONS ET COMMUNICATIONS

5.1 SYNOPSIS

Nombre de publications par année

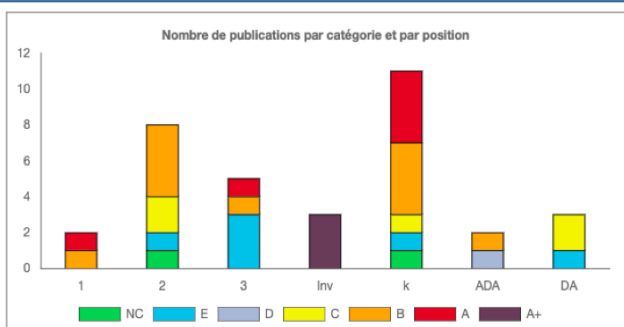
Période : 2008 - 2022	
Année	Nombre
2008	4
2009	4
2010	2
2011	1
2012	2
2013	1
2014	0
2015	2
2016	1
2017	4
2018	1
2019	1
2020	3
2021	5
2022	3
Total	34



Nombre de publications par catégorie et par position

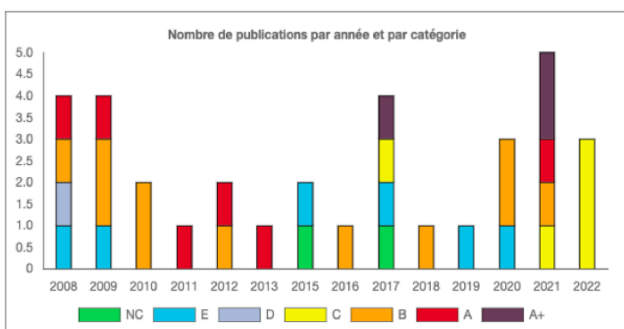
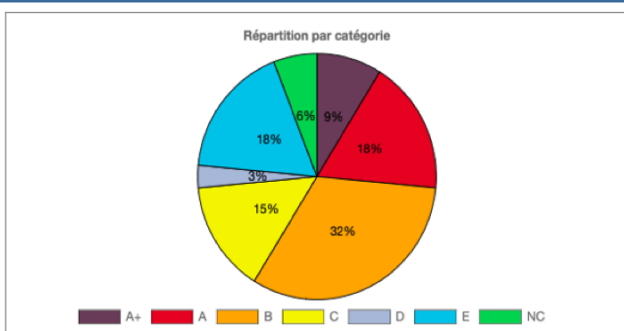
Période : 2008 - 2022								
Position	Total	A+	A	B	C	D	E	NC
1	2	0	1	1	0	0	0	0
2	8	0	0	4	2	0	1	1
3	5	0	1	1	0	0	3	0
Inv	3	3	0	0	0	0	0	0
k	11	0	4	4	1	0	1	1
ADA	2	0	0	1	0	1	0	0
DA	3	0	0	0	2	0	1	0
Total	34	3	6	11	5	1	6	2

k = Toute position intermédiaire entre 3 et ADA
 ADA = Avant Dernier Auteur
 DA = Dernier Auteur
 Inv = Investigateur



Répartition par catégorie et par année

Période : 2008 - 2022											
Année	Total	A+	A	B	C	D	E	NC	Score	Score avec Inv.	Score Frac.
2008	4	0	1	1	0	1	1	0	37	37	2.41
2009	4	0	1	2	0	1	0	0	42	42	2.23
2010	2	0	0	2	0	0	0	0	36	36	1.77
2011	1	0	1	0	0	0	0	0	32	32	1.28
2012	2	0	1	1	0	0	0	0	14	14	0.55
2013	1	0	1	0	0	0	0	0	8	8	0.42
2014	0	0	0	0	0	0	0	0	0	0	0
2015	2	0	0	0	0	1	1	0	7	7	0.60
2016	1	0	0	1	0	0	0	0	18	18	1.06
2017	4	1	0	0	1	0	1	1	19	33	1.08
2018	1	0	0	1	0	0	0	0	6	6	0.23
2019	1	0	0	0	0	0	1	0	4	4	0.24
2020	3	0	0	2	0	0	1	0	46	46	2.49
2021	5	2	1	1	1	0	0	0	18	46	0.69
2022	3	0	0	0	3	0	0	0	44	44	2.75
Total	34	3	6	11	5	1	6	2	331	373	17.80



5.2 PLAN DE PUBLICATION

5.2.1 Articles acceptés, en cours de publication

Vo Van P, Beck J, Meunier H, Venot P, Mac Caby G, Bednarek N, **Loron G**. Assessment of brain two-dimensional metrics in infants born preterm at term equivalent age. Correlation of ultrasound scans with MRI. *Frontiers in Pediatrics*, 2022.

<https://doi.org/10.3389/fped.2022.961556>

Laconi F, Bischoff C, Michelet D, **Loron G**, Bednarek N, Poli-Merol ML, Pons M, Boudaoud N. Case reports of large and symptomatic abdominal tumors in newborns: pitfalls, difficulties in management, and surgical decision making.

Accepted in: *Cancer Reports*

5.2.2 Articles soumis en cours de revue

Guittard C, Novo A, Eutrope J, Gower C, Barbe C, Bednarek N, Rolland AC, Cailles S, **Loron G**. Protocol for a prospective multicenter longitudinal randomized controlled trial (CALIN) of sensori-tonic stimulation to foster parent-child interactions and social cognition in very premature infants.

En cours de revue auprès de Frontiers in Pediatrics.

Guellec I, Ancel PY, Beck J, Loron G, Chevallier M, Pierrat V, Kayem G, Vilotitch A, Baud O, Ego A, Debillon T. Glycemia and newborns with hypoxic-ischemic encephalopathy : outcomes in the LyTONEPAL cohort.

En cours de revue auprès de Journal of Pediatrics.

Beck J, Debillon T, Guellec I, Vilotitch A, **Loron G**, Bednarek N, Ancel PY, Pierrat V, Ego A. Organizational factors associated with delayed therapeutic hypothermia in neonatal hypoxic-ischemic encephalopathy: the LyTONEPAL cohort.

En cours de revue auprès de European Journal of Pediatrics

5.2.3 Ecriture en cours

Transport et effets cérébraux du NO inhalé

Intérêt du monitoring cérébral par aEEG dans le traumatisme crânien pédiatrique

AEEG chez l'enfant : normes à partir de la technique de référence EEG.

5.3 PUBLICATIONS

1. Correction to "Hyper inflammatory syndrome following COVID-19 mRNA vaccine in children: A national post-authorization pharmacovigilance study." *Lancet Reg Health Eur*. 2022 Aug 10;100468.
2. Vo Van P, Alison M, Morel B, Beck J, Bednarek N, Hertz-Pannier L, **Loron G**. Advanced Brain Imaging in Preterm Infants: A Narrative Review of Microstructural and Connectomic Disruption. *Children (Basel)*. 2022 Mar 4;9(3):356.

3. Beck J, **Loron G**, Ancel PY, Alison M, Hertz Pannier L, Vo Van P, et al. An Updated Overview of MRI Injuries in Neonatal Encephalopathy: LyTONEPAL Cohort. *Children (Basel)*. 2022 Apr 14;9(4):561.
4. Beck J, Grosjean C, Bednarek N, **Loron G**. Amplitude-Integrated EEG Monitoring in Pediatric Intensive Care: Prognostic Value in Meningitis before One Year of Age. *Children (Basel)*. 2022 May 5;9(5):668.
5. Rozé JC, Morel B, Lapillonne A, Marret S, Guellec I, Darmaun D, et al. Association Between Early Amino Acid Intake and Full-Scale IQ at Age 5 Years Among Infants Born at Less Than 30 Weeks' Gestation. *JAMA Netw Open*. 2021 Nov 1;4(11):e2135452.
6. Pierrat V, Marchand-Martin L, Marret S, Arnaud C, Benhammou V, Cambonie G, et al. Neurodevelopmental outcomes at age 5 among children born preterm: EPIPAGE-2 cohort study. *BMJ*. 2021 Apr 28;373:n741.
7. Ouldali N, Toubiana J, Antona D, Javouhey E, Madhi F, Lorrot M, et al. Association of Intravenous Immunoglobulins Plus Methylprednisolone vs Immunoglobulins Alone With Course of Fever in Multisystem Inflammatory Syndrome in Children. *JAMA*. 2021 Mar 2;325(9):855–64.
8. Eutrope J, Novo A, Barbe C, **Loron G**, Rolland AC, Caillies S. Impact of Executive Functions and Parental Anxiety on the Development of Social Cognition in Premature Children: A Cross-Sectional Case-Control Protocol. *Front Psychiatry*. 2021;12:484571.
9. Beck J, Bednarek N, Pierrat V, Vilotitch A, **Loron G**, Alison M, et al. Cerebral injuries in neonatal encephalopathy treated with hypothermia: French LyTONEPAL cohort. *Pediatr Res*. 2021 Nov 20;
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6 PROJET DE RECHERCHE

6.1 STRUCTURE DE RECHERCHE DE RATTACHEMENT

Après une collaboration de plusieurs années en tant que chercheur associé, je suis depuis mars 2021 chercheur attaché au CReSTIC (Centre de Recherche en Sciences et Technologies de l'Information et de la Communication), équipe d'accueil EA 3804, de l'Université de Reims Champagne-Ardenne.

Son équipe MIPA (Medical Imaging Processing and Analysis) rassemble des chercheurs en analyse multimodale et traitement du signal.

Le Projet de recherche MAIA (Analyse multiphysique par imagerie pour l'étude du développement cérébral chez le prématuré), financé par une ANR, a contribué à la mise en place de nombreuses collaborations reliant le CReSTIC, le service de néonatalogie du CHU de Reims, le laboratoire du Pr Fabrice Wallois à Amiens (GRAFMC), et l'Institut Mines-Telecom Bretagne. L'élaboration du projet de recherche ci-dessous a bénéficié de ces interactions et collaborations.

6.2 THEME DU PROJET DE RECHERCHE

Ce projet a pour thème essentiel la relation entre structure et fonction au sein du cerveau en développement. C'est plus exactement, chez l'enfant, la relation entre mise en place des structures et fonction à venir. Le concept structure-fonction, ancien en neurophysiologie, a abandonné la « simple » localisation corticale des fonctions, pour évoluer vers une relation entre fonction et boucle, puis fonction et réseau (Raichle 2009). Plus récemment il apparaît même que certains réseaux ont comme spécificité... d'être relativement peu spécialisés !

Sur un cerveau mature, une lésion cérébrale va entraîner une modification de la fonction portée par cette structure au sein de son réseau. C'est, par exemple, la sémiologie classique du déficit au décours d'un thrombus artériel.

Concernant l'enfant, la relation structure-fonction est plus confuse et silencieuse.

Les altérations fonctionnelles induites par une altération de structure ne vont pas être visibles immédiatement. Dans l'expérience clinique, on observe un délai entre la constitution d'une lésion chez le nouveau-né et la potentielle symptomatologie classique déficitaire : de trois à six mois dans le cas de lésions des voies motrices, quelques années pour les atteintes pariétales qui se manifestent par des troubles des apprentissages, et parfois près d'une décade entre la lésion frontale et l'apparition d'une épilepsie séquellaire. Dès lors, si la sémiologie des déficits est prise en défaut, il faut recourir à d'autres approches sémiologiques (par exemple, l'observation des mouvements « généraux » spontanés du petit enfant) ou à des examens « paracliniques » analysant morphologie et/ou fonction.

De plus, les altérations du cerveau en développement ne sont pas seulement une lésion dans un organe, mais plutôt la modification d'une trajectoire de développement préétablie - comme on dévierait un satellite de son orbite. Les travaux de revue de Joseph Volpe l'illustrent remarquablement chez le prématuré (Volpe 2019). Il faudrait envisager les altérations du cerveau en développement de façon dynamique, évaluer l'organe et la fonction de façon séquentielle, et non réaliser une investigation à un instant « t ».

Pour aller plus loin, les processus qui altèrent la trajectoire peuvent être l'inflammation, l'ischémie, les carences en facteurs de croissance, etc... Mais aussi la nature de l'input sensoriel qui stimule la fonction : voix – ou absence de voix, mouvement ou immobilité, excès de douleur, de lumière ou de bruit...

Nous parlons ici de « plasticité ». En pratique, la plasticité est souvent présentée comme les mécanismes permettant de s'adapter et de contourner une déficience (Feuillet, Dufour, and Pelletier 2007), et une piste prometteuse de thérapie. Plus fondamentalement il s'agit de tous les processus qui rendent les processus de maturation sensibles ou dépendants de l'expérience, ils sont à ce titre également impliqués dans les altérations de développement induites par des agressions, ou des stimulations délétères (Takesian and Hensch 2013; Hübener and Bonhoeffer 2014; Lammertink et al. 2021).

La découverte des phénomènes moléculaires à l'œuvre lors de l'entrée et la sortie des phases dites « critiques » de développement ouvre la porte à des thérapies neuroprotectrices ou curatives lors de rééducations, par exemple... (Hensch 2005; Ismail, Fatemi, and Johnston 2017) Mais vu la complexité des fonctions en jeu, il faudra être capable de visualiser, comprendre, la trajectoire de développement de différentes fonctions propres à un individu, dans son contexte. Trois objectifs sont poursuivis dans ce projet : développer des outils, des marqueurs d'une trajectoire développementale ; tester le comportement de ces outils dans des pathologies-prototype ; évaluer l'impact des interventions ultra-précoces sur les marqueurs de trajectoire et le développement à long terme.

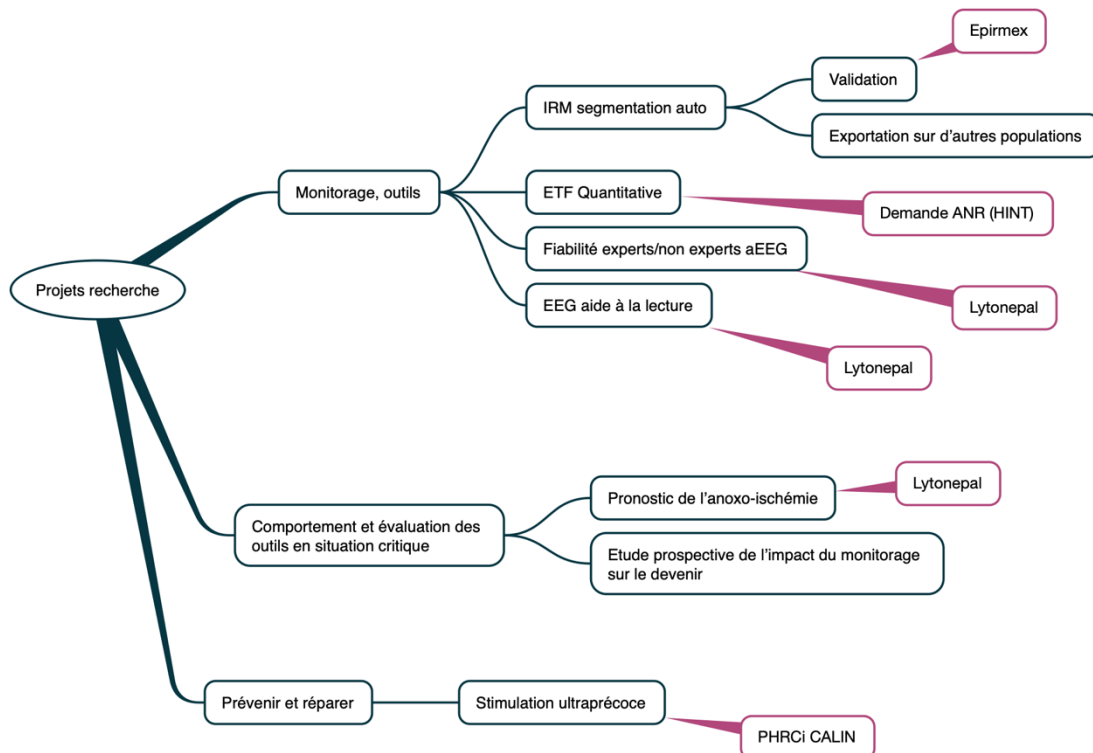


Figure 8 : Synopsis des axes de recherche et des projets s'y rattachant

6.3 SUIVRE LES ALTERATIONS STRUCTURELLES DANS UN CERVEAU EN DEVELOPPEMENT

Dans les suites du travail déjà engagé avec le CReSTIC, je poursuis la mise au point et la validation d'outils de monitoring du cerveau immature. Nous nous focalisons sur des outils non invasifs et transposables en pratique clinique.

6.3.1 Algorithme de segmentation automatique

Nous avons abordé plus haut les étapes d'élaboration d'un algorithme de segmentation automatique, basé sur des réseaux neuronaux antagonistes, le premier réseau effectuant dans le même temps hyper-résolution et segmentation, le deuxième sélectionnant les images réalistes de segmentation. Cet algorithme a été entraîné pour partie sur des images sélectionnées à partir de la base de données DHCP (Delannoy et al. 2020).

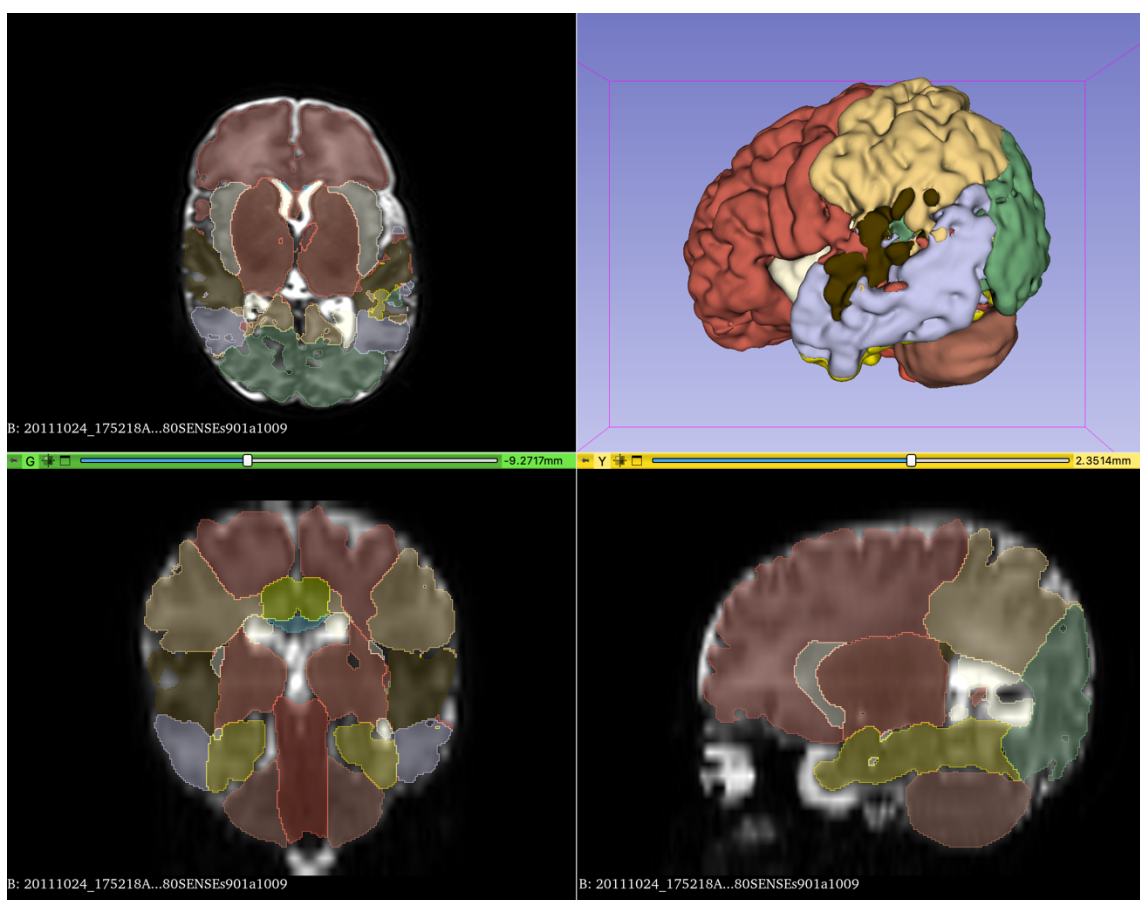


Figure 9 : Segmentation IRM SegSRGAN
IRM provenant de la banque IRM Epirmex.

Après cette phase d'entraînement, nous avançons actuellement dans la phase contrôle et de validation des résultats de segmentation obtenus sur les images de la cohorte EPIRMEX. Nous avons développé des masques et des règles, contraintes appliquées à l'algorithme avec nouvel entraînement, ou traitement d'image post-hoc. Ceci nous a permis, par exemple, d'exclure le contenu des orbites de la segmentation.

Ensuite, on ne peut s'affranchir pour ce nouvel algorithme d'une vérification de chaque segmentation régionale obtenue. Il s'agit de valider les segmentations de meilleure qualité et de comprendre les raisons d'une moins bonne segmentation. Nous souhaitons une méthode de

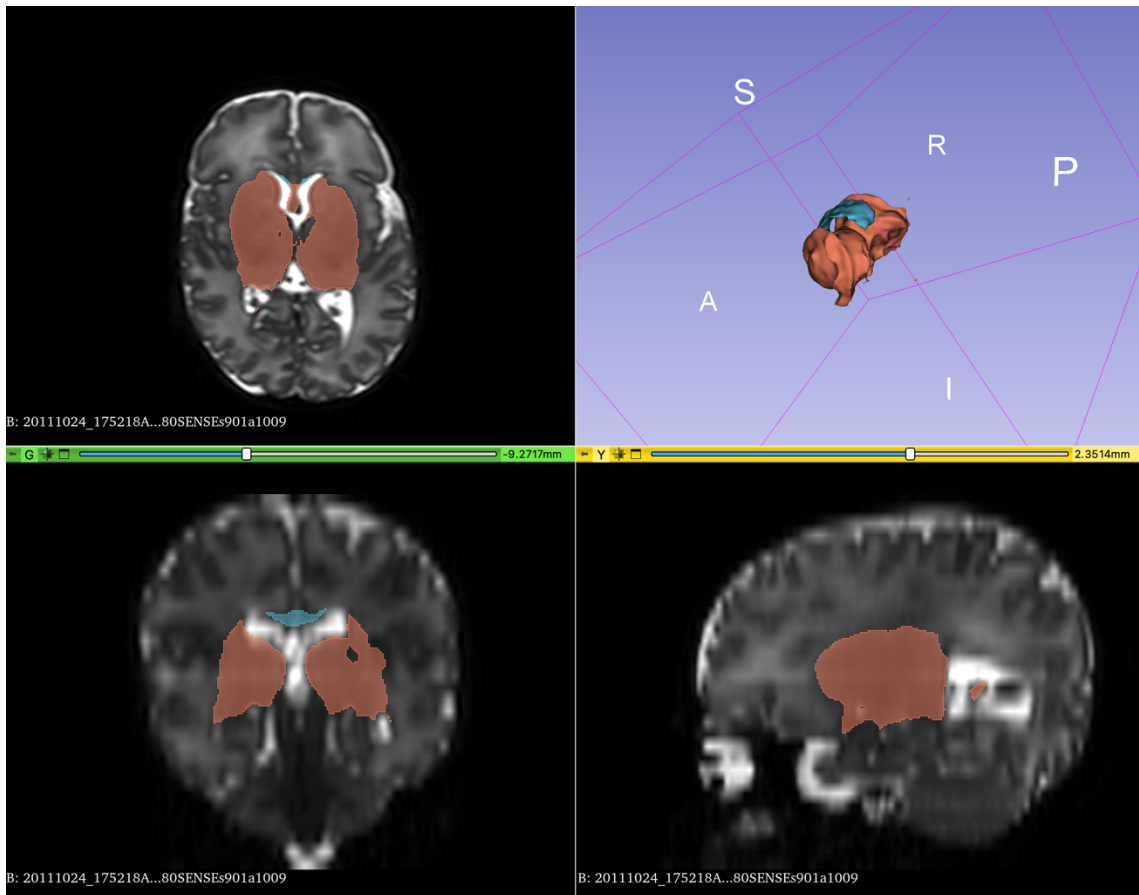


Figure 9 : Segmentation SegSRGAN : Validation de label

Exemple de label : ensemble noyaux gris centraux (rouge sombre) et corps calleux (bleu)

validation objective, reproductible, diffusable. Nous développons un score de validation, dont nous testons actuellement la performance et qui sera publié. Il s'agit pour une part d'une analyse visuelle de la qualité de la segmentation de chaque label : respect des frontières de la région à segmenter, uniformité du label. Deux relecteurs indépendants coteront ce score, label par label, IRM par IRM. Un traitement statistique des labels obtenus sera également appliqué recherchant les « aberrations mathématiques » de segmentation. Il est par exemple possible de dépister facilement les zones mal segmentées lorsque le volume obtenu est émietté, dispersé : la contiguïté et l'éloignement des voxels sont le type de données que nous analysons statistiquement.

La deuxième étape est de vérifier la « vérité » des volumes obtenus. Nous obtenons bien des volumes régionaux, mais sont-ils vrais ? Dans quel intervalle de confiance le volume obtenu correspond-il au volume de la structure « anatomique » ? Malheureusement il n'existe pas de base de données de volume cérébraux néonataux « vrais » sur des séries anatomopathologiques par exemple... Il nous faut valider ces volumes avec d'autres approches d'imagerie. La comparaison de la segmentation automatisée à une segmentation manuelle n'est pas réaliste : vu le temps nécessaire à une segmentation coupe par coupe, peu d'examen seront utilisés pour la validation. Nous avons opté pour la comparaison de mesures 2D (diamètres et

surfaces) sur certains plans, mesures réalisées sur à la main sur l'image de départ et le résultat final de la segmentation. Ces mesures 2D seront automatisables et fourniront un « contrôle qualité » automatique lors des utilisations ultérieures de cet algorithme.

6.3.2 Segmentation cérébrale et devenir

En reprenant la cohorte Epirmex nous mettrons en relation les données volumétriques des imageries cérébrales avec les données périnatales et post-natales, ainsi que les données de suivi. Nous chercherons à confirmer des atteintes structure-fonction récemment décrites, à commencer par les atteintes de la fosse postérieure. En effet, le cervelet du prématuré est particulièrement vulnérable : entre 20 et 40 (SA) il connaît une croissance majeure et se connecte avec les structures sus-tentorielles. Via des interconnexions avec les noyaux pontiques et thalamiques, il participe à la maturation corticale sus-tentorielle. Des études récentes confirment qu'il joue un rôle crucial dans le développement moteur, cognitif, social et comportemental via des effets modulateurs sur le développement cortical.

Selon une topographie précise, les lésions cérébelleuses sont associées à des défauts de croissance et d'organisation corticale secondaires, processus nommé « diaschisis neurodéveloppemental ».

Nos objectifs seront :

- 1/ De caractériser les anomalies de volume cérébelleux, leur topographie (hémisphère cérébelleux droit, gauche et vermis) et de rechercher des anomalies de volume au niveau des structures sur lesquelles les faisceaux cérébelleux projettent : noyaux gris centraux, tronc cérébral et cortex cérébral.
- 2/ D'étudier les facteurs de risque périnataux en fonction de la typologie des lésions cérébelleuses qualitatives ou quantitatives.
- 3/ D'établir enfin les corrélations entre les volumes obtenus et le devenir neurodéveloppemental tel qu'il a été rapporté dans cette cohorte, à 2 ans et 5 ans avec les questionnaires ASQ, classification GMC, MABC et échelle WISC-IV.

6.3.3 Utilisation de l'algorithme de segmentation au-delà de la cohorte EPIRMEX

Nous prévoyons de décliner l'utilisation de cette segmentation automatique pour le calcul de volumes cérébraux sur d'autres populations d'étude : les nouveau-nés à terme ayant présenté une anoxo-ischémie (PHRC LyTONEPAL, cf infra) et dans le cadre du protocole CALIN (cf infra). Au-delà, des collaborations permettront d'utiliser cette segmentation dans d'autres protocoles et de le valider sur d'autres cohortes.

L'IRM, via les traitements d'image avancés, apporte une photographie fine d'un point de la trajectoire de développement. Mais répéter ces IRM pour appréhender une dynamique est encore peu réaliste : l'outil est peu disponible en routine, et le transport d'enfants prématurés et/ou instables n'est pas possible avec des temps d'examen longs. L'échographie pourrait apporter ces informations séquentielles.

6.3.4 Traitement d'images échographiques

L'échographie est une technique facilement utilisable au chevet des nouveau-nés, répétable sans risque au cours du séjour. Plusieurs fenêtres acoustiques permettent d'exploiter différents

secteurs angulaires : fontanelle antérieure, fontanelle postérieure, écaïlle temporale, mastoïde, foramen occipital. Ses défauts principaux l'absence de plan de coupe perpendiculaire dans les trois plans (axial/para sagittal/coronal) pour comparer à l'IRM ou reconstruire des volumes, la dépendance à l'opérateur pour être reproductible, l'existence « d'angles mort » (convexité cérébrale), la résolution inférieure à l'imagerie par résonance magnétique.

Sur ce dernier point, les appareils les plus récents ont effectué d'énormes progrès (Deffieux, Demené, and Tanter 2021). En particulier le développement informatique permet une fréquence d'acquisition d'image supérieure à 1000Hz, avec une très nette amélioration de la résolution.

Nous avons montré l'augmentation de cette résolution dans l'étude des vélocimétries doppler du grand prématuré, sur ces principaux axes artériels cérébraux et dans ses deux premières semaines de vie (paragraphe 4.3.2).

A présent, comme pour l'IRM ces dernières années, il faudrait développer une analyse qualitative à partir des échographies transfontanellaires itératives.

Pour ce faire nous devons établir une base de données échographique.

Un protocole de recherche est en cours de rédaction pour déposer ce projet et constituer une base locale, avec un protocole ETF précis et une sauvegarde des examens anonymisés avec les données cliniques pertinentes. Cette collecte locale ne nécessitera pas de financement de grande ampleur, une demande d'AOL pourrait y pourvoir. Cette banque locale permettra l'étude préliminaire au dépôt d'un PHRC pour constituer une base de données de grande ampleur incluant échographie haute résolution sériées et IRM.



Figure 10 : Echographie transfontanellaire
Coupe sagittale. Acquisition haute résolution avec échographe de nouvelle génération

6.3.4.1 Croissance cérébrale appréciée sur des mesures en deux dimensions

La littérature a montré la relativement bonne corrélation entre certaines mesures 2D et certains volumes régionaux cérébraux. Notre équipe vient de le démontrer lors d'un travail de recherche original la bonne corrélation entre des mesures 2D en échographie et des mesures 2D en IRM (Vo Van, Beck, et al. 2022). Dans un premier temps, nous pourrions donc réaliser ces mesures 2D de façon séquentielle, pour établir des normes – similaires aux courbes de croissance fœtale. L'acquisition d'IRM en dehors du terme pour un échantillon de la population sera pertinente pour vérifier la corrélation à d'autres âges gestationnels que le terme.

6.3.4.2 Traitement d'image échographique

La suite du projet est d'accéder à la segmentation cérébrale à partir de données échographiques.

Plusieurs défis sont posés : la qualité des images échographiques peut être un obstacle, l'acquisition de secteurs dans différentes incidences et non un volume 3D, et la possibilité de données manquantes (« angles morts » à la convexité). Plusieurs pistes sont évoquées avec l'équipe du CRESTIC, en particulier en associant des données IRM aux données échographiques. Ce projet a été inclus dans une demande d'ANR (Allocation nationale de recherche – projet HINT) dont la lettre d'intention a été retenue, et qui figure en liste d'attente sur la liste finale.

Mesurer les volumes sur des échographies sériées permettra évidemment de suivre des vitesses de croissance, structure par structure, et de suivre la trajectoire de croissance en fonction d'abaques. Si des altérations de la vitesse de croissance sont corrélées à des déficits ultérieurs, cela ouvrirait la porte à une surveillance renforcée voire des stratégies de neuroprotection. Au-delà, suivre les volumes cérébraux de façon séquentielle aiderait à la localisation de source en électrophysiologie.

6.4 DEVELOPPEMENT DES OUTILS ELECTROPHYSIOLOGIQUES : LA SITUATION DE L'ANOXO-ISCHÉMIE A TERME.

Nous avons abordé plus haut l'intérêt du protocole LyTONEPAL pour l'étude de l'anoxo-ischémie sur un large échantillon de population (paragraphe 4.3.4).

Nous souhaitons utiliser la base de données d'examen électrophysiologiques pour plusieurs projets de recherche. Nous avons à notre disposition 765 EEG et 334 aEEG interprétés dans les CRF (LyTONEPAL principal) ; Via LyTONEPAL ancillaire nous avons les données sources aEEG pour 109 patients et EEG pour 260 patients (plus de 5300 heures d'EEG). Enfin, parmi les données EEG, nous avons près de 4000 heures en format ouvert, exploitables en traitement informatique. Les EEG et aEEG standards ont été relus par un groupe expert.

Plusieurs travaux seront développés à partir de cette base de données.

6.4.1 Vérifier la concordance des interprétations aEEG entre experts et non-experts

L'électroencéphalogramme d'amplitude est une technique réputée facile d'interprétation, en comparaison à l'EEG standard. Nous souhaitons le vérifier dans la situation de l'anoxo-ischémie à terme. Le Dr Laura Menvielle a, comme mémoire de DESC, réalisée une analyse préliminaire sur ce sujet, que nous souhaitons approfondir.

Nous rapporterons dans ce travail la concordance entre l'interprétation in situ et l'interprétation après relecture centralisée. Les items étudiés seront : la classification du tracé de fond aEEG, la détection de crises et d'état de mal électrique.

La population d'étude comportera tous les patients de l'étude ancillaire (dont les tracés aEEG et EEG ont été collectés pour relecture). L'agrément inter-observateur sera quantifiée par le test kappa pondéré. En cas d'interprétation discordante entre experts et cliniciens, les tracés seront relus par un binôme expert pour tenter de caractériser les raisons de ces discordances.

6.4.2 Dresser le panorama de l'utilisation de l'EEG en France dans l'anoxo-ischémie

L'encéphalopathie anoxo-ischémie à terme se manifeste à l'EEG par une dépression du tracé, dont la profondeur et la durée spontanées sont corrélées à la gravité des lésions et au pronostic (Bourel-Ponchel et al. 2022). L'examen clinique peut également renseigner sur la gravité du tableau (score de thompson, score de Sarnat), mais parfois avec retard. La sédation pour

ventilation mécanique rend inutilisable l'évaluation clinique. L'hypothermie thérapeutique, initiée avant 6 heures de vie, a profondément modifié le devenir de ces patients, surtout ceux dont l'atteinte est modérée. Pour toutes ces raisons l'évaluation électrophysiologie tient une grande place dans le diagnostic de gravité d'une encéphalopathie anoxo-ischémique, c'est un critère d'inclusion en hypothermie thérapeutique pour de nombreuses recommandations nationales ou internationales. Quel est, en pratique, le taux de recours à l'EEG et à l'aEEG dans cette population ? L'étude LyTONEPAL permet d'y répondre.

Sur les 736 patients inclus dans LyTONEPAL, et pour lesquels ces données sont disponibles, 720 ont bénéficié d'une exploration électrophysiologique à un moment de leur hospitalisation. Mais seulement 39.2% des patients a bénéficié d'un EEG ou d'un aEEG avant l'initiation de l'hypothermie thérapeutique. Cette analyse a été l'objet du travail de mémoire du Dr Lucas Chevalier, en option Néonatalogie dans le service du CHU de REIMS. Nous allons détailler les facteurs démographiques, institutionnels associés à la pratique de l'électrophysiologie dans ces indications. En particulier, est-ce que l'horaire d'admission influence l'accès à ce soin ? Est-ce que certains patients ne sont pas enregistrés, parce qu'ils semblent moins sévèrement atteints ? ou à l'inverse parce qu'ils le sont de façon caricaturale ? Même si le design de l'étude ne permet pas de remonter au raisonnement du clinicien, l'analyse des caractéristiques des patients nous permettra de retenir certaines hypothèses. Et vraisemblablement de proposer des actions, qu'elles soient de formation des équipes, ou encore des arguments aux organismes de tutelle pour améliorer l'accès à ces examens sur tout le territoire.

6.4.3 Exploitation des relectures expertes

Les tracés EEG dont les données brutes étaient disponibles ont été classés par période de 6 heures. Les deux dernières heures de chaque période de 6 heures ont été relues. Les électrophysiologistes, tous spécialisés dans l'EEG du nouveau-né, ont catégorisé les tracés selon la classification française et le score de Murray. De plus, ont été analysées : la durée des bouffées et leurs caractéristiques, la durée de discontinuité, la présence d'asynchronie, d'asymétrie, d'encoches frontales, de delta brushes, de dysrythmie lente antérieure, de pointes, la labilité du tracé, la présence de cycles veille/sommeil et le temps passé en crise par période de 6 heures.

Ces annotation précises du tracé nous permet plusieurs travaux :

- La comparaison de la relecture experte à celle fournie dans le CRF. Il a déjà été montré une variabilité interindividuelle importante entre relecteurs, l'ampleur de l'étude permet de la mesurer à nouveau. Plus encore, nous pourrons caractériser les raisons de ces discordances si elles sont systématiques.
- Nous pourrons ensuite redéfinir le tracé « normal » sous hypothermie. En effet, les classifications ont toutes été élaborées avant l'ère de l'hypothermie thérapeutique, or celle-ci induit des modifications EEG même sur cerveau sain. Il est important de caractériser le tracé EEG des enfants au devenir (imagerie et clinique) normal.
- De même, il faut redéfinir les anomalies préoccupantes de l'EEG. Certaines caractéristiques EEG peuvent avoir perdu de leur acuité pronostique avec l'usage de l'hypothermie. La déconstruction de la classification que nous avons effectuée lors de ces relectures centralisées nous permettra de vérifier la valeur prédictive des différents items.

A terme, l'ambition du projet LyTONEPAL est de dresser un score pronostique précoce et tardif à partir des données obstétricales, cliniques, biologiques, morphologiques et électrophysiologiques.

6.4.4 Vers une aide à l'interprétation en temps réel de l'EEG

L'électroencéphalogramme standard est le gold standard pour l'analyse de la fonction cérébrale, de façon non invasive. Il est utilisable chez le prématuré. Le corollaire de sa grande richesse est la complexité de sa relecture. Il est également très susceptible d'être artéfacté, surtout lorsqu'il est enregistré en service de soins critiques, où un grand nombre de dispositifs électriques, sources d'artéfacts, cohabitent au chevet de l'enfant.

Interpréter un EEG de nouveau-né nécessite de l'habitude, et l'agrément inter-observateur est perfectible concernant le classement des tracés de fond, voire médiocre pour l'identification de figures pathologiques (Massey et al. 2019; Stevenson et al. 2020; Kubota et al. 2020).

Il existe un besoin d'aide à l'interprétation de l'EEG, auquel un algorithme supervisé pourrait répondre. Nous formulons l'hypothèse qu'un algorithme supervisé aiderait à augmenter la reproductibilité de la relecture, et à répondre à l'augmentation de la demande d'enregistrements.

La banque de données électroencéphalographique collectée pour le volet ancillaire du projet LyTONEPAL est une opportunité pour concevoir et entraîner un algorithme supervisé. Un grand nombre d'heures d'EEG ont été catégorisées par des experts en double lecture. Bien sûr les étapes sont nombreuses avant une véritable aide à la lecture, nos premières étapes se concentrent sur l'identification des artéfacts et le classement du tracé de fond.

Nous nous appuyerons sur ces relectures pour le classement approprié des tracés qui serviront de vérité terrain pour entraînement de l'algorithme.

6.5 ÉVALUER L'APPORT DU MONITORAGE DANS LA PRISE EN CHARGE DU PATIENT

Nous avons abordé, parmi les travaux de recherche antérieurs, deux publications sur l'intérêt de l'aEEG dans des situations de détresse neurologique aiguë (paragraphes 4.3.1.2 et 4.3.1.3). Si les bénéfices rendus par cette technique de monitoring en continu semblent toujours indéniables pour les équipes qui les utilisent, il existe très peu de travaux démontrant l'impact de cette surveillance dans la prise en charge du patient.

Il s'agirait de réaliser un travail prospectif d'utilisation du monitoring aEEG en continu chez des patients de réanimation pédiatrique en situation de détresse neurologique aiguë, sous sédation. Le monitoring serait visible ou masqué pour les soignants, de façon randomisée. L'objectif principal serait de déterminer l'impact sur le devenir du patient, les objectifs secondaires concerneraient la perception des soignants de l'état neurologique du patient d'une part, et un volet médico-économique. Vu l'effectif nécessaire, un recrutement multicentrique et un appariement des patients selon la pathologie et la gravité, seraient indispensables.

6.6 PROMOUVOIR LES MECANISMES D'ADAPTATION ET DE PLASTICITE : PHRCI CALIN

Le dernier axe du projet est de proposer une intervention pendant une période sensible du développement, d'en mesurer les effets sur le neurodéveloppement, et de suivre des marqueurs morphologiques et fonctionnels du développement cérébral.

Fin 2018, j'ai obtenu le financement d'un projet de PHRC inter-régional associant les services de néonatalogie de Reims, Amiens, Nancy et Troyes. Je suis porteur de ce projet, qui a été élaboré en association avec le service de pédopsychiatrie (Dr Alexandre Novo, Pr Julien Eutrope, Pr Anne-Catherine Rolland). Nous évaluerons l'effet, chez une population d'enfants nés prématurément, d'une stimulation proprioceptive sur :

- Le développement des interactions parent-enfant
- Le développement, incluant le développement cognitif et la cognition sociale
- La symptomatologie anxiodépressive parentale post partum
- L'électrogénèse cérébrale
- La vitesse de croissance cérébrale via des échographies cérébrales séquentielles
- La structure cérébrale via IRM à terme corrigé avec tractographie.

6.6.1 Rationnel de l'étude

L'attachement se définit comme un système de comportements basé sur un processus d'expériences relationnelles, décrit chez l'animal comme l'homme (Bowlby 1983; Lorenz 1957). La base de l'attachement se construit durant les 18 premiers mois de vie du bébé - principalement les 9 premiers - et repose sur le principe que les premières interactions sont internalisées sous forme de représentations de soi et d'autrui, ainsi que de soi en relation avec autrui. Autrement dit, les premières expériences de l'enfant, sensorielles et relationnelles sont encodées dans une mémoire à long terme, et réutilisables lors de ses relations. Ces représentations issues des premières interactions définissent les Modèles Internes Opérants (MIO) qui moduleront des années plus tard les relations et émotions à l'Autre et notamment les relations intimes - amoureuses ou avec ses propres enfants (Bowlby 1982).

La grande prématurité bouleverse les interactions parents/enfant : l'enfant est séparé de ses parents, placé dans un environnement stressant, technique, potentiellement douloureux. Les comorbidités, les sédations, compromettent sa physiologie et sa disponibilité à interagir. Plus de 35 % des grands prématurés présentent des styles d'attachement non sécurisés (López-Maestro et al. 2017). De plus, à 18 mois les grands prématurés sont décrits comme plus compliants et plus passifs dans leurs modes d'interactions par rapport à la population générale (Muller and Spitz 2003). Ces modes d'interactions différents peuvent devenir des MIO propres à la personne, et être une base des interactions futures à l'adolescence et à l'âge adulte. Ce mode de relation qui diffère du développement classique peut alors être le support de troubles cognitifs (Månsson and Stjernqvist 2014).

Des interventions précoces stimulent la neuroplasticité et infléchissent positivement le développement neurologique des grands prématurés (Spittle et al. 2015). Concernant l'audition, il est démontré que les stimulations précoces ont un impact sur la gyration et l'épaisseur du cortex auditif (Webb et al. 2015).

Les soins de développement recourant aux stimulations tactiles telles que le contact peau à peau, les massages réalisés par les parents sont des possibilités d'expériences agréables à même de soutenir les interactions précoces parents/enfant.

Chez les enfants grands prématurés, la stimulation tactile de 15 minutes de massage quotidien renforce l'activité vagale, réduit le taux de cortisol, et diminue les signes de stress (Abdallah, Badr, and Hawwari 2013; Álvarez et al. 2017; Field 2016)

Chez le prématuré, les bénéfices d'un massage (stimulation sensori-tonique) précoce sur les interactions et le développement cognitif à long terme de l'enfant ne sont pas précisés, ni les mécanismes neurophysiologiques mis en jeu (Procianoy, Mendes, and Silveira 2010).

Nos objectifs sont les suivants :

- Principal : évaluer, chez les enfants nés grands prématurés, l'effet des stimulations sensori-toniques par massage associées à la technique du peau à peau réalisées par un parent sur son enfant en période néonatale sur les interactions parent/enfant mesurées à 12 mois d'âge corrigé.
- Secondaires :
 - o Évaluer, chez les enfants nés grands prématurés, l'effet des stimulations sensori-toniques par massage associées à la technique du peau à peau réalisées par un parent sur son enfant en période néonatale sur :
 - o Le développement psychomoteur évalué à 2 et 4 ans.
 - o La cognition, incluant la cognition sociale, évaluée aux 6 ans de l'enfant.
 - o Évaluer l'impact, chez les enfants nés grands prématurés, des stimulations sensori-toniques par massage associées à la technique du peau à peau effectuées par un parent sur son enfant en période néonatale sur le développement morphologique et fonctionnel du cortex sensitivo-moteur et du cervelet.

6.6.2 Méthodologie de l'étude

Il s'agit d'une étude de supériorité, randomisée, contrôlée, multicentrique, à inclusion prospective. L'étude sera réalisée en ouvert (les titulaires de l'autorité parentale et le médecin pédiatre seront informés du groupe de randomisation) mais les médecins ou psychologues réalisant l'évaluation de la qualité des interactions et la passation des questionnaires seront en aveugle du groupe de randomisation.

Les patients inclus seront les enfants nés grand prématurés (entre 25 et 32 SA) dans un des centres participants, pour lesquels les titulaires de l'autorité parentale ont accepté la participation à l'étude.

Selon les hypothèses de moyenne du score « engagement social de l'enfant » de la CIB à 12 mois de vie de $3.19 \pm 0,74$ dans le groupe « peau à peau seul » (Ruth Feldman and Eidelman 2009) et de 3.69 dans le groupe « stimulations sensori-toniques par massage associé à la technique du peau à peau », un risque alpha de 5%, une puissance de 90% et un test bilatéral, le nombre de sujets nécessaire a été estimé à 48 par groupe (NQuery 7.0). En prenant en compte un taux de perdus de vue attendu de 20%, l'inclusion de 120 enfants est prévue.

Les critères de jugement sont, pour le l'objectif principal :

- La qualité des interactions parent/enfant sera évaluée à 12 mois de vie de l'enfant (âge corrigé) par le score dans la dimension « engagement social de l'enfant » de la Coding Interactive Behavior développée par R. Feldman (CIB) (R. Feldman and Eidelman 2006).
- La qualité des interactions parent/enfant sera évaluée à 12 mois de vie de l'enfant (âge corrigé) par les scores aux 5 autres dimensions de la CIB : « sensibilité parentale », «

intrusion parentale », « émotivité négative de l'enfant/engagement de l'enfant », « réciprocité dyadique » et « états négatifs de la dyade » de la CIB.

La qualité des interactions parent/enfant sera évaluée à 6 ans de vie de l'enfant par les scores aux 6 dimensions de la CIB.

Pour les objectifs secondaires, les critères de jugement sont :

- Les symptômes d'anxiété, de dépression post-partum et de stress post-traumatique seront évalués à 7+/- 1 semaines post-natales :
 - o Via les échelles STAI, PSI, BDI, EPDS et PPQ, respectivement, chez les mères
 - o Via les échelles STAI, PSI, BDI et PCL-5, respectivement, chez les pères.
- Le neurodéveloppement et la cognition sociale, évalués à 2, 4 et 6 ans de vie de l'enfant, par :
 - o A 15 mois : BECS
 - o A 2 et 4 ans : Questionnaire ASQ, adapté à l'âge, renseigné par les parents
 - o A 6 ans : Passation de tests neuropsychologiques : WISC-V, NEPSY, TEA-CH, Épreuves de théorie de l'esprit cognitive affective.
- Étude morphologique et fonctionnelle du développement cérébral :
 - o Échographies cérébrales protocolisées dans les 72 premières heures, J7, J15 puis 1 par quinzaine jusqu'à sortie
 - o Électroencéphalographie d'amplitude : de J2 à J3 puis pendant une durée 16 heures 8 heures tous les 15 ± 2 jours
 - o Électroencéphalographie standard à J7 ± 48 heures, puis à 31 SA d'âge corrigé ± 4 jours et enfin à 35 SA d'âge corrigé ± 4 jours.
 - o IRM cérébrale sans injection avec séquences de diffusion à terme corrigé ± 15 jours

Selon le groupe de randomisation, le plan d'intervention est le suivant :

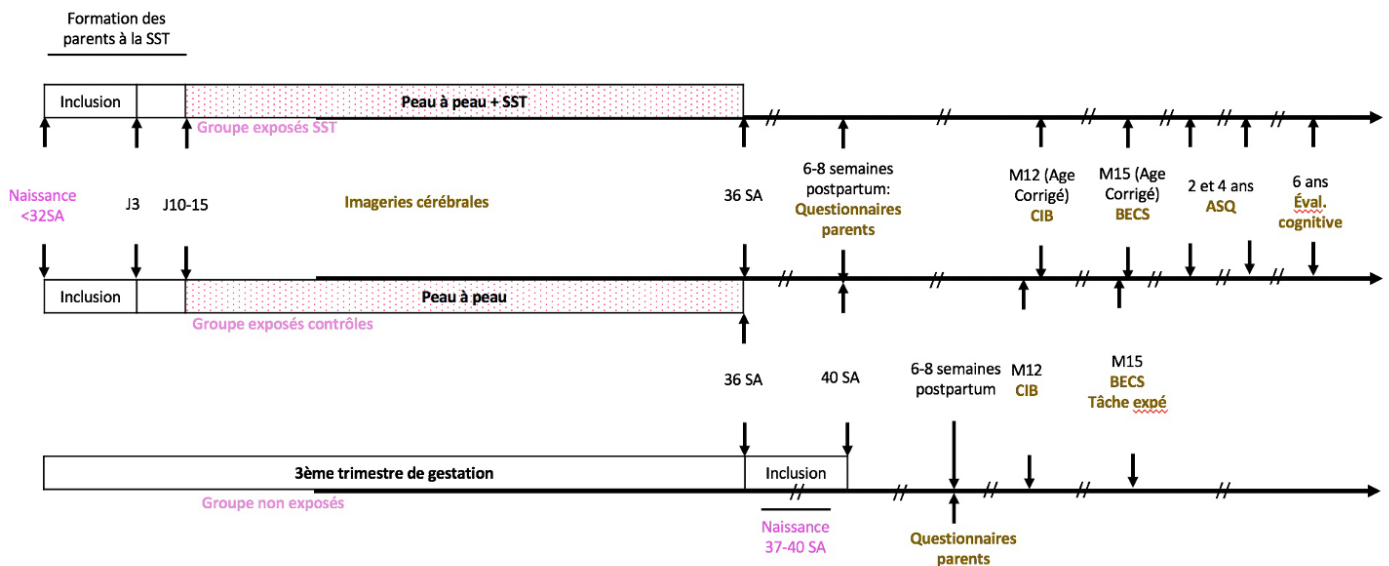
- Pour le groupe « Peau à peau et stimulations sensori-toniques »
 - o Formation d'un parent à la technique du massage par un professionnel de la périnatalité,
 - o Stimulations sensori-toniques par massage (en complément des interactions parents/enfant habituelles de type peau à peau) : dès le 10ème jour de vie et jusqu'à 36 semaines d'âge corrigé, durée de 15 minutes, 5 fois/semaine, supervisée par un professionnel de la périnatalité (psychologue ou psychomotricien).
- Pour le groupe « peau à peau seul » :
 - o Interactions parents/enfant laissées libres, encouragement au peau à peau selon le protocole du service.

Pour les évaluations de développement et de la symptomatologie de stress et anxiodépressive, la passation des échelles et questionnaires est réalisée comme suit, chronologiquement :

- Lors de la visite post-natale à 7±1 semaines, passation des échelles STAI, PSI, BDI, EPDS et PPQ pour les mères et STAI, PSI, BDI et PCL-5 pour les pères.
- Visite à 12 mois ± 1 mois à domicile avec un pédopsychiatre ou psychologue, non informé du groupe de randomisation pour évaluation de la relation enfant/parents (enregistrements

vidéo pour cotation centralisée de la CIB) et recueil de données concernant l'enfant et son environnement.

- À 15 mois \pm 15 jours (âge corrigé) : Visite dans le centre d'inclusion, avec un psychologue non informé du groupe d'inclusion, pour évaluation chez l'enfant des précurseurs du développement cognitif et cognitivo-social incluant les précurseurs à la cognition sociale, à l'aide de la batterie standardisée BECS en français
- Questionnaire ASQ renseigné par les parents à 24 et 48 mois.
- Visite dans le centre d'inclusion, à 6 ans \pm 6 mois avec un médecin pédopsychiatre ou un psychologue, non informé du groupe de randomisation pour évaluation de la cognition et notamment de la cognition sociale de l'enfant (enregistrements vidéo pour cotation centralisée de la CIB, épreuves de théorie de l'esprit cognitive et affective).



6.6.3 Résultats attendus et perspectives :

La périnatalité est une période essentielle de prévention somatique et psychique des troubles interactifs et développementaux, mais les indications et l'efficacité des outils de soins restent encore sous évaluées. Leurs liens avec le développement anatomique cérébral est quasi inexistant.

Notre étude permettrait de valider l'intérêt clinique des stimulations sensori-toniques par massage chez les enfants à risque de troubles d'interaction et donc d'attachement non sécurisé, comme le sont les grands prématurés.

De plus, notre recherche évaluerait les effets d'une intervention précoce sur la mise en place et le développement neurologique incluant la cognition globale.

Cette recherche serait la première à évaluer les relations entre stimulation sensori-tonique, mise en place des comportements d'attachement, maturation morphologique et fonctionnelle cérébrale.

Si cette étude le confirme, le soin par massage en période néonatale pourra être facilement généralisable et reproductible, y compris dans des pays dits à faible niveau de revenu.

Grâce à ce soin de prévention secondaire, nous pouvons espérer :

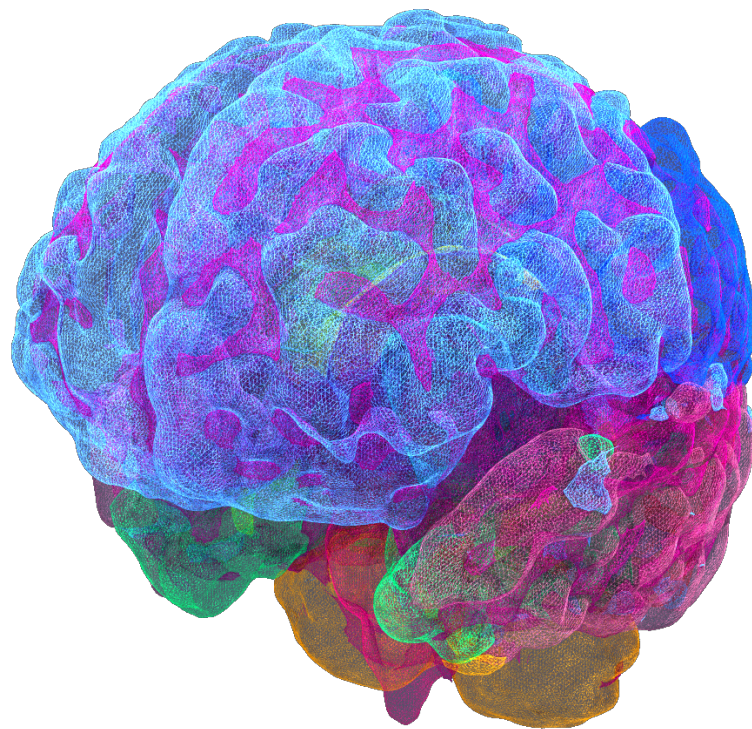
- Pour l'enfant en tant qu'individu : une meilleure capacité cognitive et sociale
- Pour la famille dans son ensemble : une diminution du niveau de stress du fait d'un attachement plus sécurisé
- Plus largement, une diminution du retentissement social de la grande prématurité.

Sur le plan fondamental, il s'agirait d'une démonstration expérimentale de l'impact de l'input sensoriel et du soutien à la parentalité sur la fonction cérébrale, en y associant potentiellement un substrat morphologique et fonctionnel

7 CONCLUSION

Le projet de recherche ici présenté conjugue trois objectifs. Tout d'abord, trouver des indicateurs d'une trajectoire de développement neurologique. Ensuite, tester la pertinence de ces indicateurs dans des pathologies emblématiques du développement cérébral – en tant que preuve de concept. Les indicateurs envisagés sont électrophysiologiques, morphologiques. Enfin, le dernier objectif est d'évaluer des interventions précoces pour tenter de corriger une trajectoire de développement.

Ce projet a été imaginé et mûri au contact de nombreux médecins et chercheurs qui m'inspirent au quotidien : au sein des services notre hôpital pédiatrique, du laboratoire du CReSTIC, des collaborations développées au-delà de notre université. Sans eux, l'aboutissement de ces travaux serait inenvisageable, qu'ils soient ici tous remerciés pour leur soutien si précieux.



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9 ANNEXES

Vous trouverez en annexe

1. Quatre articles issus du travail de thèse de doctorat
2. Les figures principales du travail de thèse du Dr Margaux Descharmes (normes aEEG enfant)
3. L'article issu du travail de thèse des Dr Jonathan Beck et Cécile Grosjean (aEEG dans la méningite de l'enfant)
4. Les figures principales du travail de Master II du Dr Hélène Meunier (Doppler des artères cérébrales chez le grand prématuré)
5. Article de revue sur l'analyse quantitative de l'IRM cérébrale du nouveau-né
6. Corrélation des mesures cérébrales en deux dimensions : IRM vs échographie
7. Le protocole du PHRCi CALIN tel que soumis à la revue : *Frontiers in Pediatrics*.

ORIGINAL ARTICLE

Nitric Oxide Plays a Key Role in Myelination in the Developing Brain

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Abstract

Inhaled nitric oxide (iNO) is one of the most promising therapies used in neonates, but there is little information available about its effect on the developing brain. We explored the effects of both iNO and endogenous NO on developing white matter in rodents. Rat or mouse pups and their mothers were placed in a chamber containing 5 to 20 ppm of NO for 7 days after birth. Neonatal exposure to iNO was associated with a transient increase in central nervous system myelination in rats and C57BL/6 mice without any deleterious effects at low doses (5 ppm) or behavioral consequences in adulthood. Exposure to iNO was associated with a proliferative effect on immature oligodendrocytes and a subsequent promaturational effect. The role of endogenous NO in myelination was investigated in animals treated with the nitric oxide synthase inhibitor N^{G} -nitro-L-arginine methyl ester (L-NAME) in the neonatal period; this led to protracted myelination defects and subsequent behavioral deficits in adulthood. These effects were reversed by rescuing L-NAME-treated animals with iNO. Thus, we demonstrate considerable effect of both exogenous and endogenous NO on myelination in rodents. These data point to potential new avenues for neuroprotection in human perinatal brain damage.

Key Words: Brain damage, Development, Myelination, Nitric oxide, Prematurity, Repair.

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This study was financially supported by INSERM, INSERM AVENIR Program, and Mairie de Paris. Drs Olivier and Loron were financially supported by Ikaria (iNOTherapeutics). The authors thank Marie Conrath, PhD, and Marie-Jeanne Brisorgueil (UMR 7101 CNRS – Université Pierre et Marie Curie, Paris, France) for their excellent scientific and technical support for the electron microscopic studies.

This experimental research program on inhaled NO is partially supported by Ikaria, Inc. However, Ikaria controlled neither the experimental protocol nor the procedures used in this study.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jneurosci.com).

INTRODUCTION

Brain injury in the premature infant is a problem of major importance, as recently emphasized by EPICure and EPIPAGE population studies (1, 2). Because of major advances in neonatal intensive care, nearly 85% to 90% of these infants now survive. Approximately 10% of the survivors later exhibit cerebral palsy, especially spastic diplegia, and an additional 25% to 50% exhibit cognitive, attentional, and/or behavioral deficits that result in failure to succeed in school. This has a major effect on the ethics of resuscitation of the most immature babies and on the economic and subsequent medical needs of handicapped infants and adults. The neurologic disabilities observed relate in considerable part to cerebral white matter injury, and preventing this injury requires an understanding of its pathogenesis. A combination of hypoxia-ischemia and maternal-fetal infection/inflammation is considered to play an important role in these processes (3). Nitric oxide (NO), which modulates vascular tone, reperfusion, inflammation, and oxidative stress, is a key component of these risk factors (4, 5).

Despite considerable advances in our understanding of the pathophysiology of brain damage during development, therapeutic options are still extremely limited. Developing new drugs for very preterm infants is currently a time-consuming and speculative strategy, with many regulatory constraints. Investigating the neurological effects of drugs that are already in common use may be a faster and more pragmatic option.

Although its use is controversial, inhaled NO (iNO) is one of the most commonly used therapies in neonatal intensive care units. Nitric oxide is thought to have only a local effect that is limited to pulmonary vascular tone and has been proposed for treatment of pulmonary hypertension-related hypoxemia and for preventing chronic lung disease. However, considerable clinical evidence suggests that iNO could also affect the developing central nervous system (CNS) (6, 7). In this study, we explored the neurological effects of iNO on the immature brain and demonstrate the major effects of exogenous (inhaled) and endogenous NO on myelination in rodents.

MATERIALS AND METHODS

Animals and Treatment Procedures

All experiments were carried out in compliance with the ethical rules of INSERM. Mother rats (Sprague-Dawley;

Janvier, Le Genest-St-Isle, France) or C57BL/6 mice (8) and their pups were placed in a normoxic, normocapnic gas chamber containing either 5 or 20 ppm of NO for postnatal days (P) 0 to 7. The concentrations of NO and NO₂ (lower than 1 ppm) were monitored using the iNOvent system (INOTherapeutics, Clinton, NJ). To determine the specific effects of iNO on rat pups, the mothers were changed daily from exposed to unexposed experimental groups. Control and iNO-exposed animals were treated similarly in 2 identical Plexiglas chambers in the same room. After the exposure periods, rat pups were maintained in the same room under strictly similar conditions.

To investigate the effect of either endogenous NO production or exogenous iNO on the developing brain, we analyzed myelination in neonatal rats treated with the inhibitor of NO synthases (NOSs), *N*^(G)-nitro-L-arginine methyl ester (L-NAME; 15 mg/kg twice a day given intraperitoneally from P0 to P7).

Immunohistochemistry

We studied 6 to 10 pups in each experimental group in 3 separate experiments. Immunolabeling with the primary antibodies (Table, Supplemental Digital Content 1, <http://links.lww.com/NEN/A141>) was visualized using the streptavidin-biotin-peroxidase method as previously described (9). Antibodies NG2 and O4 (the latter was a generous gift from Dr P.A. Rosenberg, Boston, MA) were used as markers of immature and proliferating oligodendrocytes. Antibodies to adenomatous polyposis coli (APC) and myelin basic protein (MBP) were used as mature and myelinating oligodendrocytes markers. Olig2 was used as a marker of all oligodendrocytes. To assess the specificity of Olig2 for oligodendrocyte lineage cells, double labeling using anti-glial fibrillary acidic protein (GFAP) and Olig2 was performed (Figure, Supplemental Digital Content 2, <http://links.lww.com/NEN/A142>). Cells positive for GFAP did not express Olig2, consistent with a recent report (10). For NG2 and O4 immunolabeling, rat pups were perfused transcardially with 4% paraformaldehyde in phosphate buffer (0.12 mol/L, pH 7.4). The brains were processed as previously described (9).

Quantitation of Immunoreactive Cells and Fibers

Immunoreactive cells were counted in subcortical white matter (+2.16 to -0.36 mm from the bregma) in animals killed on P3, P7, and P14. Cells were counted within a 0.25-mm² grid in at least 4 sections per animal (n = 6 or more per group).

The optical density (OD) of MBP+ fibers was measured in the cingulum in coronal sections because of the white matter thickness in this area. At least 4 sections each from 6 to 10 animals per group that were killed on either P10 or P14 were examined. Optical densities were measured at 100× magnification using a computerized image analysis system (ImageJ; NIH, MA, <http://rsb.info.nih.gov/ij/>), as previously described (11). Nonspecific background density was measured at each brain level in an area devoid of MBP immunostaining and was subtracted from the values from the cingulum.

Western Blot

Membrane proteins were extracted from the forebrain cortex, including the white matter, from P14 rat pups. Ex-

traction was achieved by homogenization in HEPES buffer containing protease inhibitors (Sigma, St Louis, MO), according to the manufacturer's instructions. Protein samples (50 μg) were incubated overnight with antibodies either MBP (Santa Cruz Biotechnology, Santa Cruz, CA), platelet-derived growth factor-α (PDGFRα; Santa Cruz Biotechnology), semaphorins 3A (Sema3A) and 3F (Sema3F; Abcam, Inc, Cambridge, MA) diluted 1:5000, or an anti-α-actin antibody (Santa Cruz Biotechnology) diluted 1:10,000. Western blot experiments were run in triplicate.

TUNEL Staining

In P7 and P21 animals, dying cells in the white matter were detected using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, as previously described (9). Stained cells were counted in 4 sections each from a single hemisphere of at least 6 animals per treatment group.

Ultrastructural Morphology of Cingulate White Matter

For electron microscopy, 100-μm-thick sagittal brain sections were cut on a vibratome, postfixed with 1% osmium tetroxide, dehydrated, and incubated in araldite. Silver-stained ultrathin sections of the cingulate white matter were made using an ultramicrotome (LKB, Bromma, Sweden), after cutting a semithin section to verify the level. Ultrathin sections were collected on copper grids. They were counterstained and examined using an electron microscope (LEO 912; Carl Zeiss, Le Pecq, France).

Quantitative Real-time Polymerase Chain Reaction

DNA-free total RNA from the brain cortex including white matter was obtained at P3, P7, P10, and P14 using a protocol adopted from Chomczynski and Sacchi (12). To standardize gene expression across samples, we first compared the expression levels of 4 housekeeping genes within the samples. For reverse transcription (RT), we used 600 ng of total RNA and the Iscript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Real-time polymerase chain reaction (PCR) was set up with Supermix (Bio-Rad) containing SYBR green for 50 cycles with a 3-step program (11). Each reaction was run twice with at least 6 animals per group; in both cases, samples were assessed in triplicate. The applied primers for real-time PCR are listed in Table, Supplemental Digital Content 3, <http://links.lww.com/NEN/A143>.

Behavioral Assessments

Open Field and Habituation

An open field consisted of a 63 × 63-cm² arena divided into 49 equal-sized squares (9 × 9 cm²) constructed of opaque Plexiglas with 40-cm-high walls and a clear Plexiglas lid. On day 1, each rat was placed in the field facing a corner for 5 minutes. The behavior of the rat was recorded using an overhead digital camera. Behavioral measures extracted from the digital video recordings illustrated the number of times the rats crossed the square sections. To minimize the stress incurred by the presence of an object during subsequent object exploration tests, on day 2, each rat was returned to the same arena with a single object for 3 minutes.

Nonspatial and Spatial Object Recognition Tests

On day 3, each rat was returned to the same arena for a 3-minute exploration period, with 2 objects located at diagonally opposite corners. One hour later, each rat was returned to the arena for a 3-minute exploration period with a familiar and a novel object located in the same corners as during the initial exploration period. On day 4, each rat was returned to the same arena for a 3-minute exploration period with 2 objects located at diagonally opposite corners. One hour later, each rat was returned to the arena for a 3-minute exploration period with the same objects but with the position of 1 object changed to another corner. During each exploration period, the time spent exploring each object was measured. Each behavioral assessment test was run with a least 18 animals per group.

Statistical Analysis

All data were reported as mean \pm SEM. Either 1- or 2-way analysis of variance was performed with age and groups as the factors, and the Newman-Keuls post hoc test was used. Statistical tests were run on GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA).

RESULTS

Inhaled NO Promotes Myelination in Neonatal Rodents

We first investigated the effect of iNO on serum concentrations of nitrites and nitrates, 2 of the main metabolites of NO. Inhaled NO induced a dose-dependent increase in serum concentrations of nitrites and nitrates (Figure, Supplemental Digital Content 4, <http://links.lww.com/NEN/A144>). We next determined whether iNO affected myelination under basal conditions. Exposure to both 5 and 20 ppm of iNO was associated with up to 40% increase in myelin formation in the developing white matter (Figs. 1A, B). Increases were observed at both P7 and P14, suggesting that the effect of iNO on myelination in normal rat pups continued after the treatment was stopped. It seemed to be transient, however, because no further differences were observed at day 21 (Fig. 1B). Upregulation of both MBP protein and gene transcription was detected just after iNO exposure at P14 (Figs. 1C, D). Inhaled NO was also associated with a transient increase in the density of mature (APC+) oligodendrocytes at P14 in the white matter (Figs. 1E, F). These results were not modified when mothers were changed daily from exposed to unexposed experimental groups, indicating that these results were directly related to effects of iNO on the rat pups. The ultrastructural features of white matter myelin were similar in iNO-exposed and controls (Figure, Supplemental Digital Content 5, <http://links.lww.com/NEN/A145>). We did not observe any differences within the cortical plate between iNO-exposed and control pups ($n = 6$ per group) using the neuronal markers RT-97 for axons and γ -aminobutyric acid (GABA) or calcium-binding protein for GABAergic neurons and interneurons (Figure, Supplemental Digital Content 6, <http://links.lww.com/NEN/A146>). Thus, in contrast to its effects on developing oligodendrocytes, iNO did not seem to induce changes in neuronal maturation.

To determine whether the effects on myelination were observed in other rodents, we also examined C57BL/6 mice at similar stages of brain development. The effects of iNO in a myelin proteolipid protein–green fluorescence protein mouse at P10 were comparable to those in P14 rat pups, that is, both proteolipid protein and MBP densities were increased in the lateral corpus callosum of the striatum in iNO-exposed mouse pups (Figs. 2A, B). The myelin fiber ODs were also increased in the white matter and basal ganglia (Fig. 2C).

These findings were sex-independent and were not related to the exposure of the dam to iNO. Exposure to iNO was not associated with any changes of the performances observed in 3 separate behavioral tests, including open field and object exploration tests performed in adult rats (data not shown).

Because 5 ppm of iNO was associated with myelination enhancement in both rat and mouse pups, all further experiments were performed using this low concentration, which is comparable to that used clinically in human neonates.

Inhaled NO Increases the Density of Proliferating Immature Oligodendrocytes in Neonatal Rats

To determine whether iNO acts through preoligodendrocyte proliferation or maturation, we first explored the effects of iNO on proliferating cells in white matter. Exposure to iNO was associated with a significant increase versus controls in the proportion of proliferating (Ki67+) cells ($p < 0.05$; Fig. 3A). Inhaled NO also induced a small but significant increase in the total number of Olig2+ oligodendrocytes (Fig. 3B). The density of immature oligodendrocytes was assessed using NG2 and O4 markers; it induced a significant increase of both NG2+ and O4+ oligodendrocytes (Figs. 3C, D). The distribution of O4+ and NG2+ cells seemed to be similar in treated and controls. The density of TUNEL+ Olig2+ double-positive cells in rat pups was also similar to that in controls throughout the hemispheric white matter (Fig. 3E). Similarly, TUNEL+ Olig2+ cell densities were not different in iNO-exposed and control groups at P21 (Figure, Supplemental Digital Content 7, <http://links.lww.com/NEN/A147>). These data strongly suggest that iNO acts first as an enhancer of oligodendroglial proliferation and that this is likely responsible, at least in part, for its effects on myelin content and APC+ cell density, that is, the newly formed oligodendrocytes induced by iNO did not seem to die.

Transcriptional Effects of iNO on Factors Involved in Oligodendroglial Lineage and Myelination

By quantitative RT-PCR analysis, iNO was associated with increased PDGFR α expression at P3 and P10 (Fig. 4A). Quantitative analysis of PDGFR α protein expression in rat pup using Western blot also demonstrated a significant increase iNO-exposed compared with controls (Figure, Supplemental Digital Content 8, <http://links.lww.com/NEN/A148>). In contrast, Sox10 expression was not affected by iNO (Fig. 4B), and other transcription factors (i.e. Nkx2.2, CREB, c-jun) were not altered at any time point in animals exposed to iNO (data not shown).

Semaphorins 3A and 3F act as axonal guidance cues and chemotactic factors for oligodendroglial cells in the developing CNS. Semaphorin 3A usually exerts a repulsive

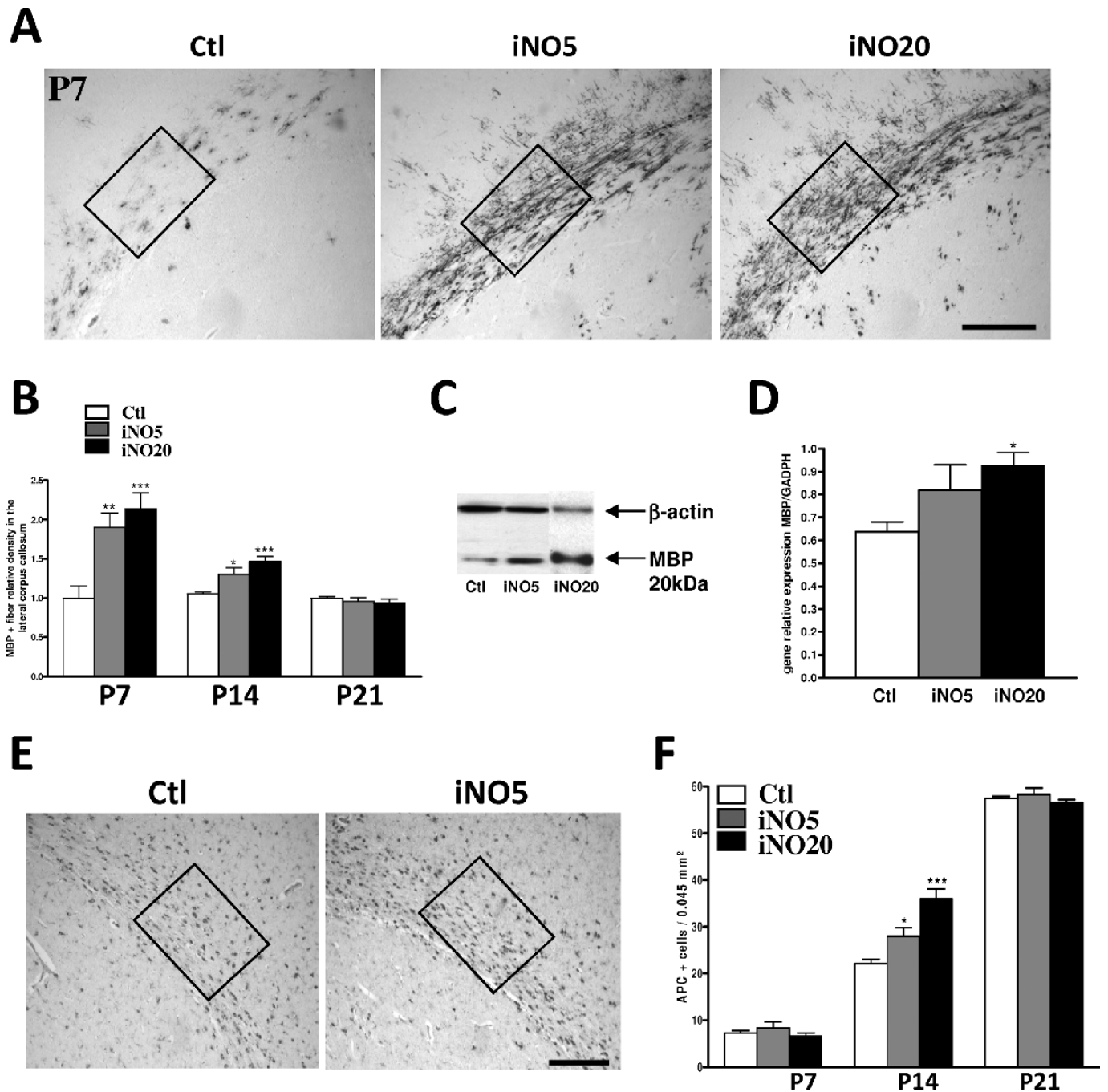


FIGURE 1. Inhaled NO enhances myelination in the developing rat brain. **(A)** Myelin basic protein+ oligodendrocytes in the lateral corpus callosum in coronal sections from control (Ctl) and rat pups exposed to 5 and 20 ppm of NO (iNO5 and iNO20) at P7. Boxes delineate the regions in which ODs were measured. Scale bar = 100 μ m. **(B)** Optical density quantification of MBP staining in the lateral corpus callosum of iNO-exposed rat pups (normalized to the respective controls) at P7, P14, and P21. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; iNO-treated groups versus controls. **(C)** Western blot shows a dramatic increase of MBP synthesis in iNO-exposed rats at P14. **(D)** Reverse transcription–PCR shows modulation of MBP transcript levels relative to glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) housekeeping gene levels after iNO exposure at P14. *, $p < 0.05$; iNO-treated groups versus controls. **(E)** Photomicrographs showing mature APC+ oligodendrocytes in the lateral corpus callosum of control (Ctl) and NO-exposed rat pups (iNO5) at P14. Bar = 100 μ m. **(F)** Quantification of APC+ oligodendrocytes in the lateral corpus callosum of control (Ctl) and iNO-exposed rat pups (iNO5 and iNO20) at P7, P14, and P21. *, $p < 0.05$; ***, $p < 0.001$; iNO-treated groups versus controls.

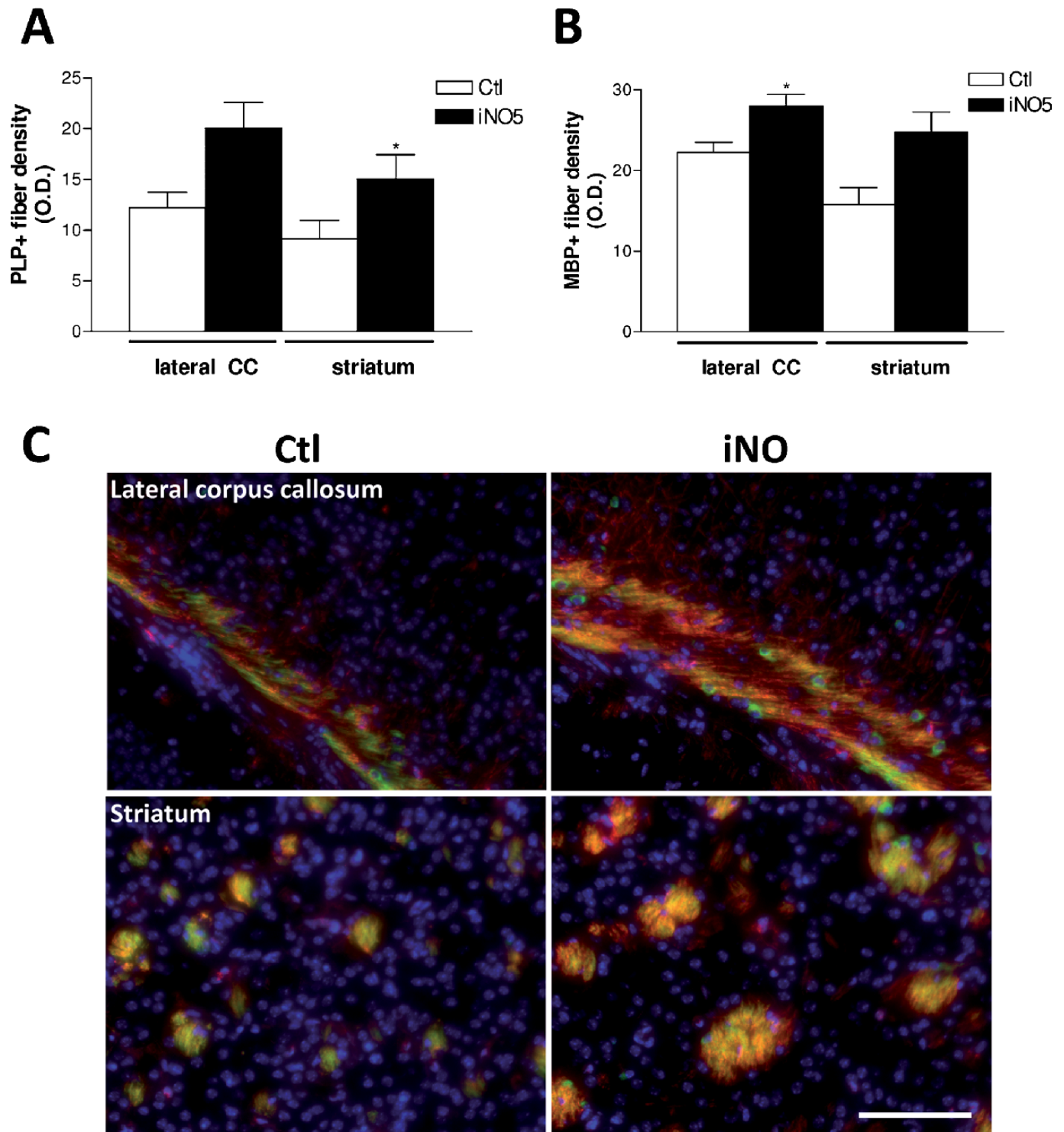


FIGURE 2. Inhaled NO enhances myelination in the developing brain of C57BL/6 mice. **(A)** Optical densities of myelin proteolipid protein (PLP) green fluorescence protein (GFP) in the lateral corpus callosum and striatum of control and iNO-exposed C57BL/6 pups at P10. *, $p < 0.05$; iNO-treated groups versus controls. **(B)** Optical density of MBP in the lateral corpus callosum and striatum of control and iNO-exposed C57BL/6 pups at P10. *, $p < 0.05$; iNO-treated group versus controls. **(C)** Proteolipid protein (green) and MBP (red) double-immunoreactive oligodendrocytes in the lateral corpus callosum and striatum of control (Ctl) and iNO-exposed C57BL/6 mice (iNO) at P10. Bar = 100 μm .

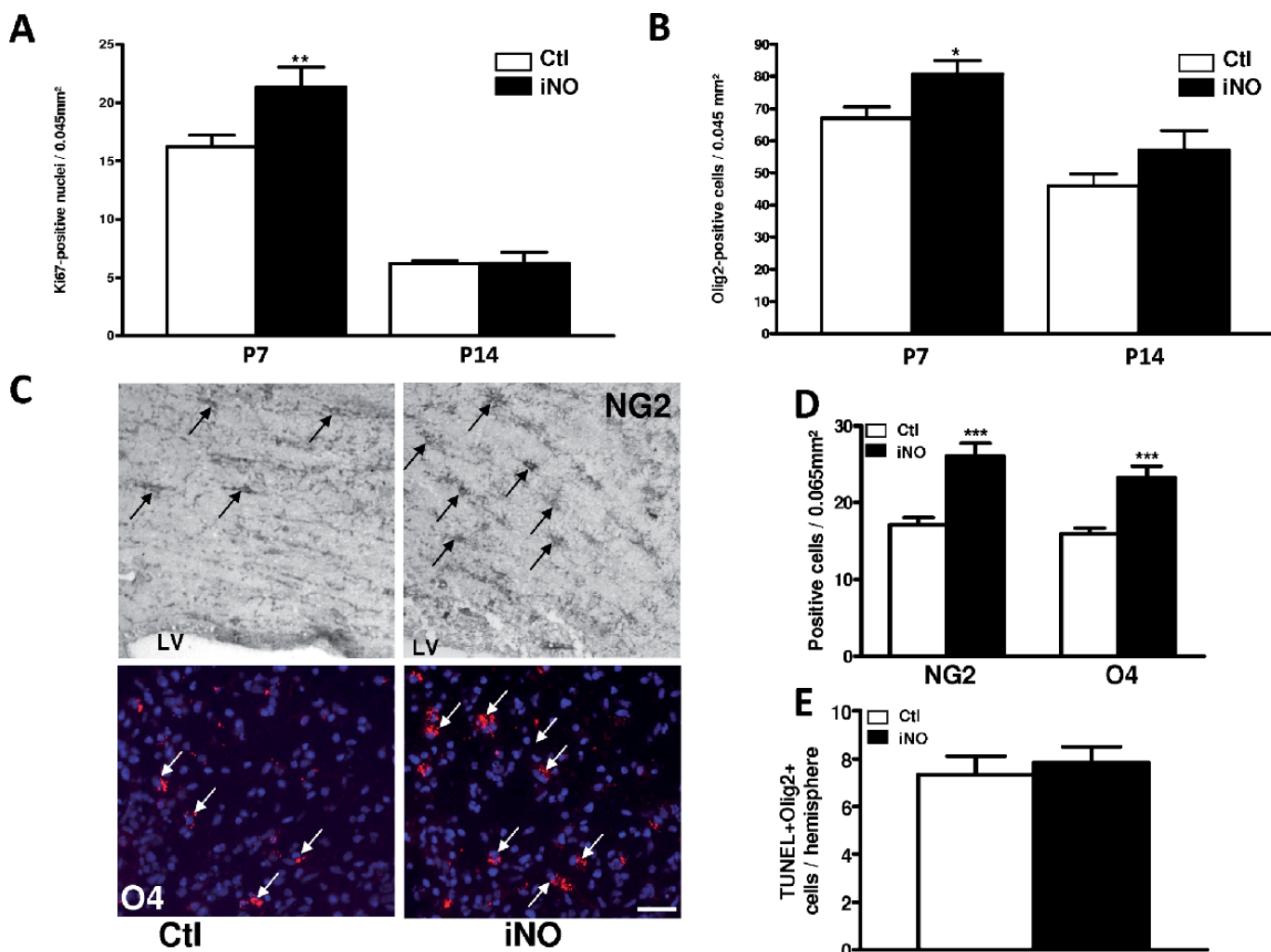


FIGURE 3. Inhaled NO promotes oligodendroglial cell proliferation in vivo. **(A)** Quantification of Ki67+ cells in the lateral corpus callosum of control (Ctl) and iNO-exposed rat pups at P7 and P14. **, $p < 0.01$. **(B)** Quantification of Olig2+ oligodendrocytes in the lateral corpus callosum of control and iNO-exposed rat pups at P7 and P14. *, $p < 0.05$. **(C)** NG2+ and O4+ oligodendrocytes in white matter from control and iNO-exposed rat pups at P7. Bar = 50 μ m. **(D)** Quantification of NG2+ and O4+ cells in the lateral corpus callosum of control and iNO-exposed rat pups at P7. ***, $p < 0.001$. **(E)** Quantification of TUNEL+ Olig2+ oligodendrocytes in the hemispheric white matter from control and NO-exposed rat pups at P7.

effect, whereas *Sema3F* exerts an attractive effect on oligodendrocyte precursors during migration (8, 13). Exposure to iNO did not alter *Sema3A* transcription on either P7 or P14, whereas *Sema3F* transcription was upregulated (Figs. 4C, D). Western blot analyses confirmed that iNO exposure induced no change in *Sema3A* protein level, whereas it was associated with increased *Sema3F* protein content on P7 (Figure, Supplemental Digital Content 8, part B, <http://links.lww.com/NEN/A148>).

Inhaled NO Reverses the Myelination Impairment and Behavioral Deficits Induced by NOS Inhibition

To determine whether endogenous NO has a role in normal brain myelination, we analyzed myelination in neonatal rats treated with the NOS inhibitor L-NAME. The concentra-

tion of nitrites and nitrates markedly decreased after L-NAME administration and was restored to normal levels by treatment with 5 ppm of iNO (Fig. 5A). L-NAME administration did not delay the initiation of myelin production in the lateral corpus callosum but significantly reduced myelin density at P14 in both the corpus callosum and striatum (Figs. 5B, C). In contrast, iNO administered to L-NAME-treated rat pups was associated with a complete restoration of normal myelination throughout the brain. The myelin impairment induced by the lack of endogenous NO production was transient. Adult rats subjected to L-NAME during the neonatal period had similar myelin content to that in controls (data not shown). However, behavioral assessments in adulthood demonstrated a dramatic effect of neonatal L-NAME treatment, that is, there was a significant deficit in reactions to novelty, either in the form of object replacement or in a new spatial location for a familiar

object (Fig. 5D). Conversely, L-NAME-treated rat pups rescued with iNO did not show any difference in performance in adult behavioral tasks in comparison to controls. Open field sessions did not reveal any significant differences between experimental groups. In contrast to its effect on myelination, L-NAME treatment did not alter calcium-binding protein+ interneuron density in the cortex (Figure, Supplemental Digital Content 9, part A, <http://links.lww.com/NEN/A149>). Similarly, GABA+ cells density was not altered by L-NAME in the cortex (data not shown). However, L-NAME did induce a small but significant decrease in GFAP+ astrocyte density in the developing white matter that was reversed by iNO (Figure, Supplemental Digital Content 9, part B, <http://links.lww.com/NEN/A149>).

DISCUSSION

We provide several lines of evidence that both exogenous (inhaled) NO and endogenous NO are involved in

myelination of the developing rodent brain white matter. This effect, even if transient, seems to be affect cognitive behavior in adulthood. Our data show that NO may act as a regulator of some genes involved in the maturation of the oligodendroglial lineage and in CNS myelination.

It is well established that NO is a physiological mediator in the CNS. Within the brain parenchyma, NO is produced from arginine and molecular oxygen by NOS expressed by glial and endothelial cells. Despite some degree of specificity, each cell type is capable of expressing all NOS isoforms, indicating the crucial role of NO during brain development. Although the role of NO in the developing brain remains poorly understood, it seems to be involved in the regulation of cerebral blood flow and in memory acquisition. After brain injury, NOS activity may be upregulated, and NO may be produced in excess, such as in excessive stimulation of glutamate receptors or the transcriptional effect of cytokine release (14). In cases of ischemia-reperfusion, there is a marked increase in NO production during the phase of reperfusion and reoxygenation

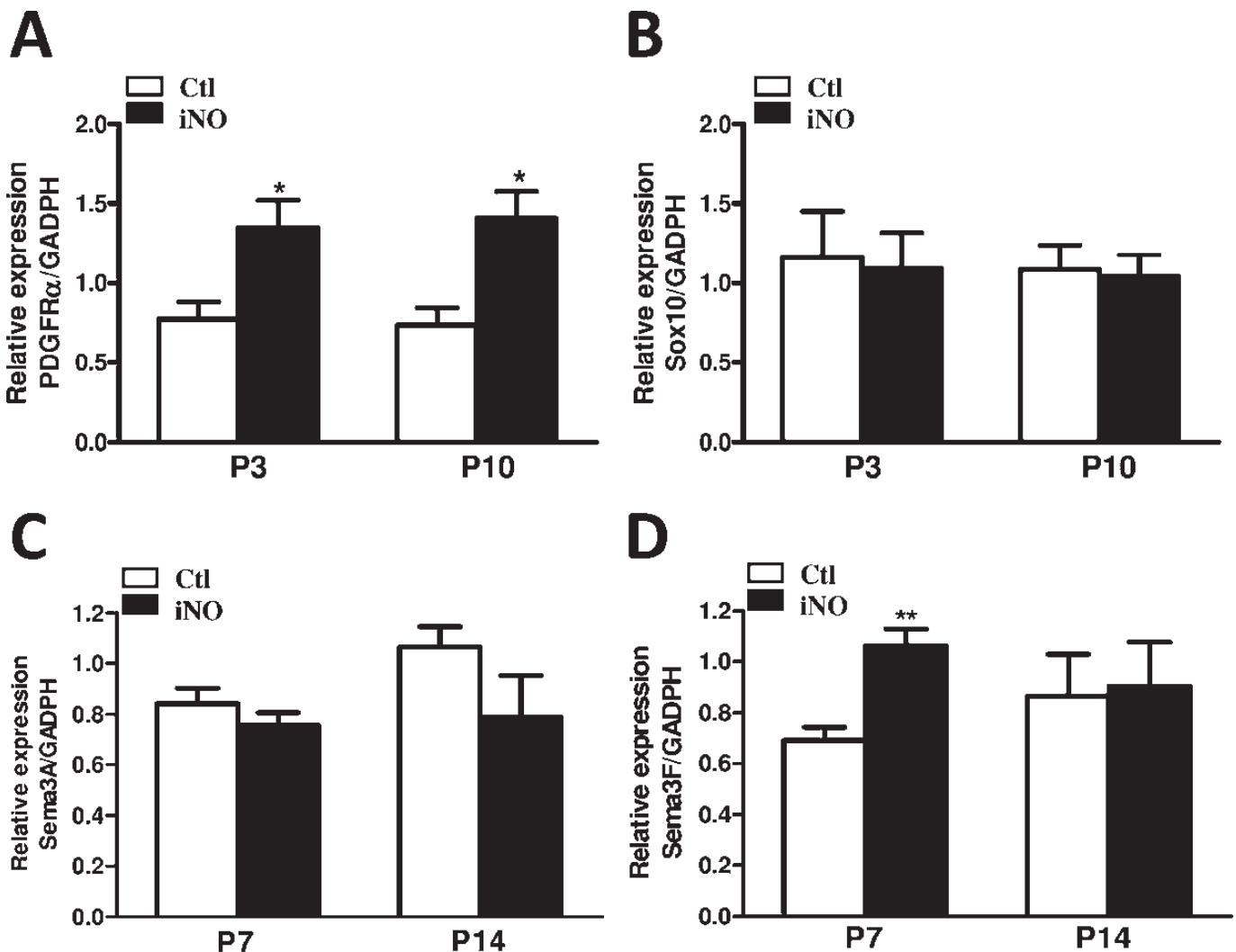


FIGURE 4. Effect of iNO on transcription of factors involved in oligodendroglial maturation or myelination. Quantitative PCR showing modulation of PDGFR α (**A**), Sox10 (**B**), Sema3A (**C**), and Sema3F (**D**) transcript levels after iNO exposure. Levels are expressed relative to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). *, $p < 0.05$; **, $p < 0.01$.

that is correlated with the intensity of the ischemic and hypoxic insult (15). Nitric oxide accumulation depends on the brain region, that is, it is particularly noticeable in the cortex, hip-

pocampus, hypothalamus, amygdala, and substantia nigra (16). Lesions of the hippocampus induced by medial cerebral artery occlusion in the rat correlate with tissue NO concentrations

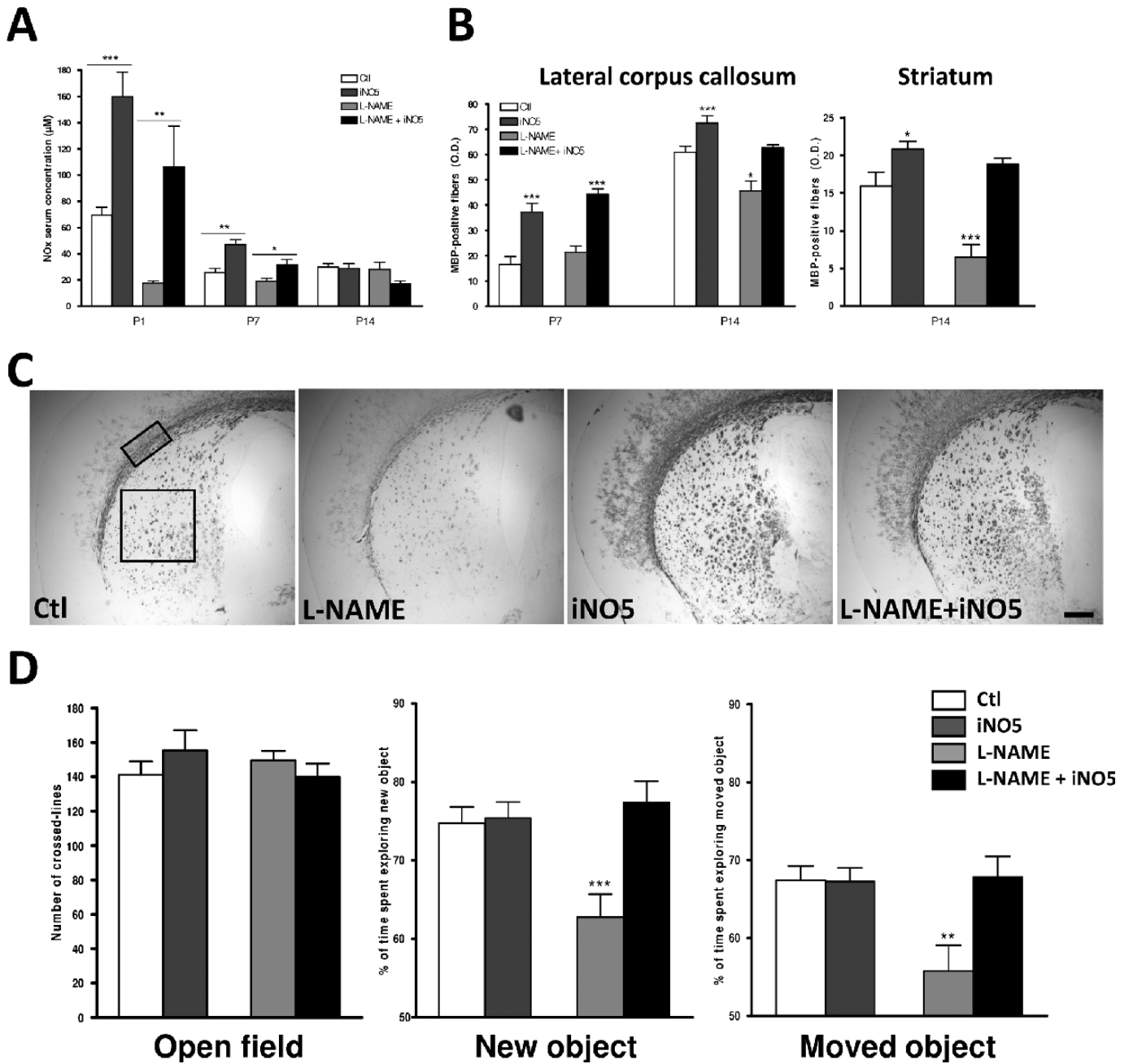


FIGURE 5. Endogenous NO production is needed for normal myelination and behavior in rats. **(A)** Quantification of nitrite and nitrate serum concentration in rat pups treated with L-NAME alone or with iNO exposure compared with control (Ctl) animals at P1, P7, and P14. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. **(B)** Optical density quantification of MBP immunoreactivity in the lateral corpus callosum and striatum of control and L-NAME-treated (alone or with iNO) rat pups at P7 and P14. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. **(C)** Myelin basic protein+ oligodendrocytes in the lateral corpus callosum and striatum in coronal sections from control and L-NAME-treated (alone or with iNO) rat pups at P14. Boxes delineate the areas in which OD was measured **(B)**. Bar = 100 µm. **(D)** Quantification of 3 behavioral tasks (i.e. open field, exploration of new object, and exploration of moved object) in adult rats subjected to L-NAME, iNO, or L-NAME + iNO during the first week of life compared with controls ($n = 18-24$ for each group). When time spent in exploring either a new or a moved object is 50% or less, this indicates a total failure to recognize change in the object. **, $p < 0.01$; ***, $p < 0.001$.

(17), and glial cells contribute to NO overproduction after ischemia through inducible nitric oxide synthase (18).

Numerous studies have demonstrated the deleterious effects of NO accumulation in brain ischemia-reperfusion (19, 20). Hypoxia-ischemia results in inflammation, especially in the developing white matter, and high NO concentrations and peroxynitrite produced locally by activated microglia may become toxic to neurons and immature oligodendrocytes in vitro (21). In contrast, NO also induces beneficial effects, probably as a function of the intracellular redox state (22). For example, an increase in brain infarct volume has been reported in sheep and rats when NO production is decreased by NOS inhibitors (23). The extent of the lesions after medial cerebral artery occlusion is reduced in endothelial cell NOS-deficient mice (24), whereas secondary neuronal damage induced by prolonged ischemia is lessened in inducible NOS knockout mice (25). The protection conferred on the ischemic brain by NO seems to be linked to vasodilation, which improves cerebral blood flow and hinders the formation of microthrombi in capillaries (26). Moreover, the nitration or nitrosylation by NO of iron- or thiol-containing enzymes may alter their biological actions by minimizing the generation of reactive oxygen species and associated oxidative stress (27–29). Several studies have demonstrated beneficial effects of NO in iron-related brain injury in vitro and in vivo (30). In addition, the less reactive NO radical scavenges highly reactive oxygen species and converts them into nonradicals such as nitrates and nitrites. Inhaled NO can scavenge thiyl radicals, converting them into biologically active *S*-nitrosothiols, which have a longer half-life in vivo. Among them, the endogenous *S*-nitrosylated reduced glutathione (GSNO) is approximately a 100-times more potent than glutathione itself (31). Finally, NO inhibits brain lipid peroxidation both in vivo and in vitro (29, 30) through the scavenging of lipid peroxyl radicals and thiol radicals. Moreover, GSNO may be an endogenous NO reservoir that can release NO when it reacts with either Cu⁺ or thioredoxin (32–35). Freshly prepared GSNO not only produces NO-like biological effects but also protects against oxidative stress in the endothelium, myocardium, and brain tissue (30, 31, 36, 37). *S*-nitrosylated reduced glutathione is also capable of detoxifying peroxynitrite in vitro, which explains why peroxynitrite is a weak pro-oxidative agent in the brain in vivo and in vitro.

How iNO affects myelination and behavior in adulthood remains unclear. We found here that transient myelin changes observed with L-NAME were associated with long-term behavioral alterations. These results suggest that a transient delay in myelination during the early postnatal period could lead to long-term behavioral and cognitive consequences in adult. Because we did not identify abnormal neuronal differentiation in young and adult rats subjected to NOS inhibitors, we speculate that dysmyelination could be responsible for the behavioral impairment observed in treated animals. Indeed, it is remarkable that iNO was protective of myelination during the first 2 weeks of life associated with and subsequently improved cognitive tasks. However, there is little evidence in the literature to correlate behavioral assays to changes in myelination, and conclusions should therefore be made with caution, but animals with myelin dysregulation have been investigated

using the same behavioral assays as used in the present study (38, 39). In these reports, reduction in myelination and dysmyelination led to marked deficiencies in memory and locomotor activity.

In addition to its effect on oligodendroglial cell proliferation and maturation, we demonstrated that expression of semaphorins might also play a role in this process. We found an imbalance between *Sema3A* and *Sema3F* (mostly due to an increase in *Sema3F* expression) associated with the transiently increased white matter myelin content in the iNO-exposed rat pups. Recently, *Sema3F* has been recognized to be not only an axonal guidance molecule but also an attractant for oligodendroglial precursors and subsequent myelinating oligodendrocytes (8). Moreover, oligodendroglial precursors expressed class 3 semaphorins receptors, that is, neuropilins (NRP1 and NRP2) and Plexin B3 (40–42). Involvement of neuropilins and semaphorins in orchestrating the migration patterns of developing oligodendrocytes may explain the recruitment of NG2/O4⁺ oligodendrocytes in the developing white matter we observed in response to iNO exposure. Although the distribution of these developing oligodendrocytes throughout the developing white matter seemed to be similar in iNO-exposed pups and controls, the question of the distribution of younger oligodendroglial progenitors could be altered in iNO-exposed pups remains unanswered. In addition to the *Sema3F* transcriptional regulation induced by iNO, a direct effect of the NO/cyclic guanosine monophosphate (cGMP) signaling pathway on class 3 semaphorins properties cannot be ruled out. Indeed, NO synthesis and the subsequent activation of guanylyl cyclase to produce cGMP induce the conversion of *Sema3A* repulsion to attraction (43).

Inhaled NO is currently a therapy used worldwide in sick full-term and preterm neonates. In addition to the well-known vasodilatory properties of NO, several recent experimental studies have suggested a possible role for the vascular endothelial growth factor/NO pathway in restoring pulmonary angiogenesis and enhancing distal lung growth (6). In contrast, the effect of iNO on CNS development remains controversial. For many years (because NO was known to increase bleeding time and inhibit platelet aggregation), it was feared that iNO would increase the incidence of intracranial hemorrhage in critically ill preterm neonates (44). Later, clinical studies demonstrated that there was no significant increase in intracranial bleeding in preterm neonates (45). More intriguing and exciting was the fact that iNO seemed to decrease the risk of cognitive impairment by 47% in exposed preterm infants observed at 2 years (7).

Recently, the pathogenesis of cerebral white matter injury in premature infants has been revisited (3). Cerebral white matter injury is characterized by the loss of premyelinating oligodendrocytes and is associated with a high risk of neurodevelopmental impairment. Here, we showed that iNO induces an increased proliferation of oligodendroglial progenitors, suggesting that iNO could be a candidate for preventing white matter injury. The unique cerebrovascular anatomy and physiology of the premature baby underlies the exquisite sensitivity of the white matter to the abnormal milieu of preterm extra-uterine life and especially to ischemia-reperfusion promoted by impaired cerebral autoregulation and inflammation. The

generation of reactive oxygen species may trigger excitotoxicity and the attack of premyelinating oligodendrocytes by free radicals because of the immaturity of antioxidant enzyme systems and iron accumulation. Again, our study raises the possibility that intracerebral NO concentration might be a mechanism of defense against oxidative stress injury in immature oligodendrocytes (22).

In conclusion, both endogenous and exogenous NO play a crucial role as an effector molecule in the myelination of the developing CNS, and iNO may have effects on the CNS as well as on the lungs in exposed patients. Our data also suggest that iNO could be a potential candidate for neuroprotection in perinatal white matter lesions.

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Neuroprotective Effect of Inhaled Nitric Oxide on Excitotoxic-Induced Brain Damage in Neonatal Rat

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Abstract

Background: Inhaled nitric oxide (iNO) is one of the most promising therapies used in neonates. However, little information is known about its impact on the developing brain submitted to excitotoxic challenge.

Methodology/Principal Findings: We investigated here the effect of iNO in a neonatal model of excitotoxic brain lesions. Rat pups and their dams were placed in a chamber containing 20 ppm NO during the first week of life. At postnatal day (P)5, rat pups were submitted to intracranial injection of glutamate agonists. At P10, rat pups exposed to iNO exhibited a significant decrease of lesion size in both the white matter and cortical plate compared to controls. Microglia activation and astrogliosis were found significantly decreased in NO-exposed animals. This neuroprotective effect was associated with a significant decrease of several glutamate receptor subunits expression at P5. iNO was associated with an early (P1) downregulation of pCREB/pAkt expression and induced an increase in pAkt protein concentration in response to excitotoxic challenge (P7).

Conclusion: This study is the first describe and investigate the neuroprotective effect of iNO in neonatal excitotoxic-induced brain damage. This effect may be mediated through CREB pathway and subsequent modulation of glutamate receptor subunits expression.

Citation: Pansiot J, Loron G, Olivier P, Fontaine R, Charriaud-Marlangue C, et al. (2010) Neuroprotective Effect of Inhaled Nitric Oxide on Excitotoxic-Induced Brain Damage in Neonatal Rat. PLoS ONE 5(6): e10916. doi:10.1371/journal.pone.0010916

Editor: Kenji Hashimoto, Chiba University Center for Forensic Mental Health, Japan

Received: March 11, 2010; **Accepted:** May 7, 2010; **Published:** June 1, 2010

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Funding: The experimental research program of Dr. Olivier Baud and his coworkers concerning iNO is partially supported by Ikaria, Inc. This study was financially supported by the INSERM, the INSERM AVENIR Program and the Mairie de Paris. Paul Olivier and Gauthier Loron were financially supported by Ikaria (iNOtherapeutics). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The experimental research program of Dr. Olivier Baud and his coworkers concerning iNO is partially supported by Ikaria, Inc. However, this does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

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Introduction

Brain injury in the premature infant is a problem of major importance. Approximately 10 percent of the survivors from very preterm birth later exhibit cerebral palsy (CP) and an additional 25 to 50 percent exhibit cognitive, attentional, and/or behavioral deficits. These neurologic disabilities observed relate in considerable part to cerebral white matter injury [1]. Factors that seem involved in the pathophysiology of CP in these models include hypoxia and ischemia, infection and inflammation, excitotoxicity, accumulation of reactive oxygen species, and deficiencies in growth factors [2,3]. These factors seem to act in combination to cause damage to the developing white matter.

Glutamate accumulation may be a mechanism common to many risk factors for CP. Glutamate, the major excitatory neurotransmitter, acts via several groups of receptors, namely, N-methyl-D-aspartate (NMDA) receptors, alpha-3-amino-hydroxy-5-methyl-4-isoxazole (AMPA) receptors, kainate receptors, and metabotropic receptors (mGluRs). Excessive activation of glutamate receptors may cause cell vulnerability, in part as a result of intracellular calcium influx [4,5]. Intracerebral injection of

glutamate agonists into the neocortex and white matter of newborn rodents produces histological lesions that mimic the brain damage observed in preterm neonates [6–8]. In addition to excitotoxicity, nitric oxide is recognized as being a key modulator of risk factors involved in CP, by regulating vascular tone, reperfusion, inflammation and oxidative stress [9,10].

Despite considerable advances in our understanding of the pathophysiology of brain damage during development, therapeutic options are still extremely limited. Inhaled nitric oxide (iNO) is one of the most commonly used therapies, promising but also controversial, in neonatal intensive care units. This molecule is thought to have only a local effect, limited to the vascular tone of the lungs, and has been proposed to treat pulmonary hypertension-related hypoxemia and chronic lung disease. However, increasing experimental and clinical evidences suggest that iNO could also have an impact on the developing central nervous system [11,12].

Here, we describe the neuroprotective effect of iNO in neonatal excitotoxic-induced brain damage. This effect appears to be mediated through pAkt-pCREB pathway and subsequent modulation of glutamate receptor subunits expression.

Materials and Methods

Ethics statement

Full details of the study have been approved by Robert Debré research council review board; the approval number is 2009-02. All experiments were carried out in compliance with the ethical rules of INSERM.

Animals and model of excitotoxic brain lesions

Twenty-four hours before delivery, pregnant female rats (Sprague-Dawley, Janvier, Le Genest-St-Isle, France) were placed in a normoxic, normocapnic gas chamber containing either 5 or 20 ppm inhaled NO and <1 ppm NO₂ for postnatal days (P) 0 to 7. The concentration of NO and NO₂ was monitored using the iNOvent system (INOTherapeutics, Clinton, NJ).

P5 rat pups of both sexes were used for this study. Ibotenate (Tocris, Bristol, UK), NMDA (Tocris) and S-Willardiine (Tocris) were diluted in phosphate buffer saline (PBS). Ibotenate activates NMDA and metabotropic glutamatergic receptors while S-Willardiine activates both AMPA and kainate receptors. Ibotenate (10 µg), NMDA (4 µg) or S-Willardiine (15 µg) was injected intracerebrally on P5 to rat pups, as previously described [6]. Briefly, rat pups anesthetized with isoflurane were kept under a warming lamp to maintain body temperature. They were injected intracerebrally (into the neopallial parenchyma) on P5. Intracerebral injections were performed with a 26-gauge needle on a 50 µl Hamilton syringe mounted on a calibrated microdispenser. The needle was inserted 2 mm below the external surface of skin. The tip of the needle was placed in the frontoparietal area of the right hemisphere, 2.5 mm from the midline in the lateral-medial plane, and 4 mm from the bregma in the rostro-caudal plane. It was confirmed by histopathological observation that the tip of the needle always reached the periventricular white matter. Two 1 µl boluses of ibotenate, NMDA or S-Willardiine were injected at 20 second intervals. The needle was left in place for an additional 20 seconds.

In a first set of experiments, P5 rat pups were intracerebrally injected with PBS. Pups treated with intracerebral PBS injections had minimal lesions, mostly consisting of needle tracks, as previously reported [13]. Therefore, control animals were kept into room air and treated animals were exposed to iNO.

At least 12 animals of each treatment group were killed by decapitation 5 days after the injection, and the brains were processed as previously described [14]. In all the experiments described below, two investigators blinded to treatment group determined the size of the lesion in each pup.

Determination of lesion size

Rat pups were sacrificed by decapitation 5 (P10) or 25 (P30) days after the excitotoxic challenge. Brains were fixed immediately in 4% formalin and remained in this solution for 5 days. Following paraffin embedding, we cut 16-µm thick coronal sections. Every third section was stained with cresyl-violet. The size of neocortical and white matter lesions can be defined by the length on three orthogonal axes: the lateral-medial axis (in a coronal plane), the radial axis (also in a coronal plane, from the pial surface to the lateral ventricle), and the fronto-occipital axis (in a sagittal plane). In previous studies [6,14], we found an excellent correlation among the measurements from the three axes of the excitotoxic lesions. Based on these findings, we cut serial sections of the entire brain in the coronal plane for this study. This permitted an accurate and reproducible determination of the sagittal fronto-occipital diameter (which is equal to the number of sections where

the lesion was present multiplied by 16 µm). We used this measure as an index of the lesion volume.

Immunohistochemistry

In each experimental group, we studied 6 to 10 pups in three separate experiments. Immunolabeling with the primary antibodies listed in File S1 was visualized using the streptavidin-biotin-peroxidase method as previously described [15].

Immunoreactive cells were counted in the white matter underlying the cortex (+2.16 to −0.36 mm from the bregma) in animals sacrificed on P10. Cells were counted within a 0.25 mm² grid, in at least 4 sections per animal, and 6 or more animals per group.

Optical density of pCREB-positive cells

The optical density of pCREB-immunoreactive cells was measured in the cortical plate in coronal sections (+2.16 to −0.36 mm from the bregma). At least 4 sections each from 6 to 10 animals per group, sacrificed on either P1 or P7, were examined. Optical density was measured at 20× magnification using a computerized image analysis system (ImageJ, NIH, MA, <http://rsb.info.nih.gov/ij/>). Nonspecific background density was measured at each brain level in an area devoid of pCREB immunostaining, and subtracted from the values for the cortex.

Quantitative real-time PCR

DNA-free total RNA from the brain cortex including the white matter was obtained using a protocol adapted from Chomczynski and Sacchi [16]. Quantitative RT-PCR was used to assess gene expression for VEGF, VEGF-R1 and 2 and for glutamate receptor subunits GluR1–4, NR1, NR2A, NR2B, NR2C and NR2D and mGluR1–8. To standardize gene expression across samples, we first compared the expression levels of four well-known house-keeping genes within the samples. For reverse transcription, we used 600 ng of total RNA and the Iscrip cDNA synthesis kit (Bio-Rad, Hercules, CA). Real-time PCR was set up with Supermix (Bio-Rad) containing syber green for 50 cycles with a three-step program. Each reaction was run twice with a least 6 animals per group, and in both cases, samples were assessed in triplicate. The applied primers for real time-PCR have been previously reported [17].

Western Blot

Membrane proteins were extracted from forebrain cortex, including white matter, taken in P1 and P7 rat pups. Extraction was achieved by homogenization in Hepes buffer containing protease inhibitors from Sigma, according to the manufacturer's instructions. We loaded 50 µg of protein from each sample were incubated overnight with either a Akt, pAkt, pCREB, ERK1/2 or pERK1/2 antibody or an anti-α-actin antibody. Western blot experiments were run in triplicate with at least 4–6 animals (see File S1).

ELISA assays

We used specific ELISA assays from R&D system Europe (Lille, France) to quantitate VEGF and cAMP Response Element Binding Protein (CREB) phosphorylated at S133 levels in the whole brain samples according to the manufacturer's instructions.

Statistical analysis

All data were reported as means ± S.E.M. Analysis of variance was performed with age and groups as the factors, and the Newman-Keuls post-hoc test was used. Statistical tests were run on

GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA).

Results

Effect of iNO on brain lesion induced by intracranial injection of various glutamate agonists

Rat pups injected on P5 with ic ibotenate, NMDA or S-Willardiine developed cortical lesions and periventricular white matter cysts in all cases. The cortical lesion was typically characterized by neuronal loss in all neocortical layers and almost complete disappearance of neuronal cell bodies along the axis of excitotoxin injection. In P5 rats, iNO 20 ppm induced a significant neuroprotection of both the cortical plate and the developing white matter against ibotenate- or NMDA-induced lesions (Figure 1A, C–D); in contrast, iNO induced a moderate neuroprotection of the cortical plate but not white matter of S-Willardiine-induced brain lesions when observed on P10 (Figure 1A). The protective effect of iNO was dose-dependent as iNO at 5 ppm induced a non significant trend to decrease the cortical and white matter lesion size in excitotoxic-induced lesions (Figure 1B). No significant neuroprotective effect was detected when rat pups were exposed to iNO only after the excitotoxic challenge. No difference was observed between male and female pups.

Because maximum neuroprotective effect was observed when ibotenate injection was associated with iNO 20 ppm, further

experiments were conducted using this excitotoxin and iNO concentration. In the developing white matter, iNO 20 ppm reduced ibotenate-induced astrogliosis (GFAP) and microglial activation (ED-1) when analyzed 5 days after the insult. Glial cells counts were performed within external capsule around white matter cyst (see Figure 1C). To analyze cell types in the glial scars in the injured white matter, mature activated astrocytes density was assessed by counting GFAP-positive cells with number of processes and enlarged cell bodies in P10 rat pups (Figure. 2 A–B). This density was found significantly higher in pups exposed to room air compared to those exposed to iNO. Because ic injection of excitotoxins was associated with infiltration of monocytes-macrophages around white matter lesion, we measured microglial cells density using ED-1, a marker for macrophages [13]. Activated microglial cells density was found significantly lower in iNO-treated animals (Figure 2C). When compared to control animals, iNO failed to increase the density of total population of oligodendrocytes around the white matter lesion; similarly, iNO was unable to improve the density of cells labelled with APC, a marker of mature oligodendrocytes (Figure 2D).

Effect of iNO on glutamate receptors subunits gene expression

Because excitotoxicity and genetic regulation of glutamate-receptor expression are known to play a key role in brain damage [17], we investigated whether glutamate-receptor gene expression

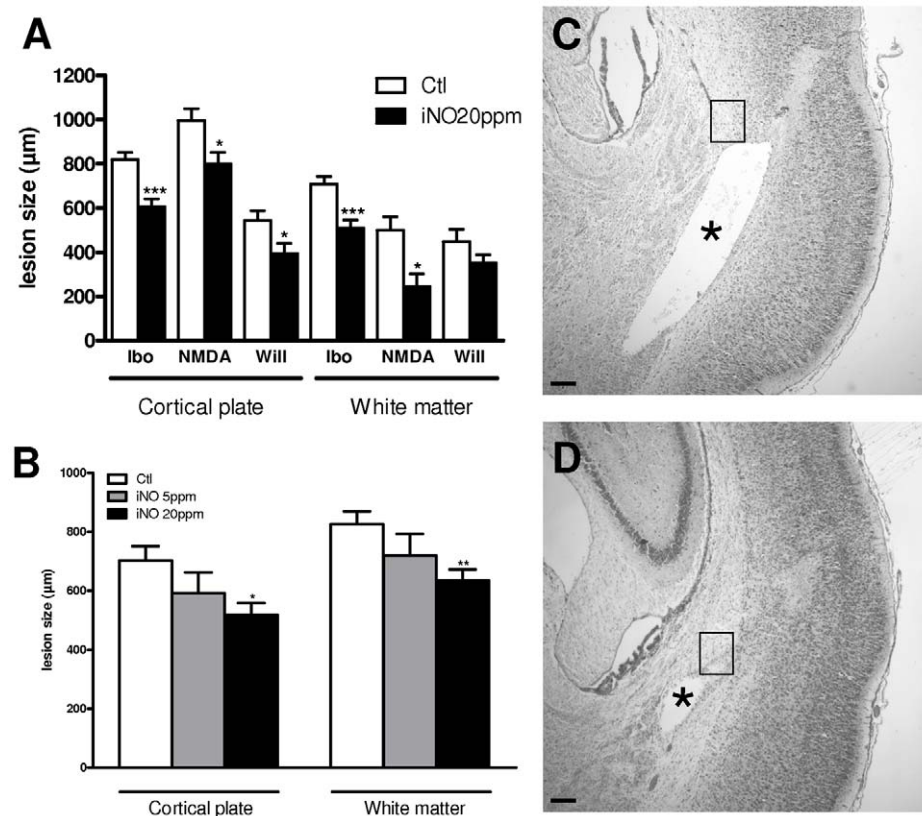


Figure 1. iNO confers neuroprotection in excitotoxic-induced brain lesion in neonatal rat. A. Quantitative analysis of lesion size in cortical plate and white matter induced by ic injection of either ibotenate (ibo), NMDA or S-Willardiine (will) in P5 rat pups with and without iNO exposure (** $p < 0.001$; * $p < 0.05$). B. Dose-dependence of the neuroprotective effect of iNO at 5 and 20 ppm in rat pups subjected to ic injection of ibotenate (** $p < 0.01$; * $p < 0.05$). C–D. Cresyl violet–stained sections showing brain lesions induced by ibotenate intracerebrally injected on P5 and studied at the age of P10. iNO exposure reduced ibotenate-induced cortical lesion and white matter cyst (asterisk). Bar = 50 µm. Boxes delineate the regions in which glial cell counts were performed.

doi:10.1371/journal.pone.0010916.g001

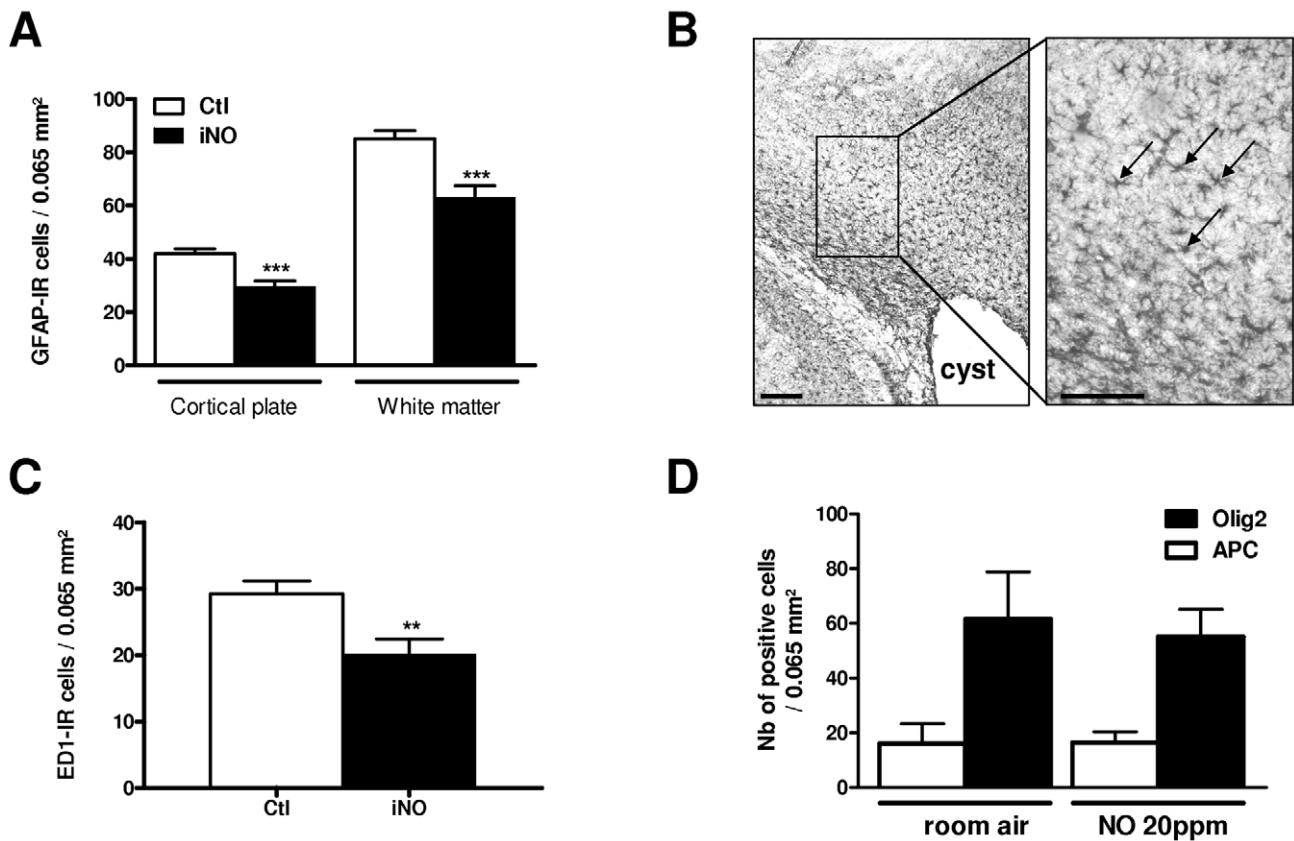


Figure 2. iNO reduces glial reaction around excitotoxic-induced brain lesion in neonatal rat. A: Quantitative analysis of GFAP-positive cells density in P10 rat pups with and without iNO exposure in cortical plate and white matter (***) $p < 0.001$). B: Coronal sections showing GFAP+ cells within white matter in room air-exposed P10 rat pups (controls). Activated mature astrocytes displayed number of processes and enlarged cell bodies (arrows). Bars = 50 μ m. C: Quantitative analysis of ED1-positive cells density in P10 rat pups with and without iNO exposure around the white matter lesion (**) $p < 0.01$). D: Quantitative analysis of Olig2- and APC-positive cells density in P10 rat pups with and without iNO exposure around the white matter lesion.

doi:10.1371/journal.pone.0010916.g002

was altered by iNO, and may account for the neuroprotective effect of iNO. We assessed the expression of glutamate-receptor subtypes using quantitative real-time PCR demonstrating a downregulation of most of them just before the intracranial injection of glutamatergic agonist in P5 rat pups. Significant 1.5 to 2-fold downregulation was observed for AMPA/kainate-receptor subunits GluR1 and 4, the NMDA receptor subtypes NR1, 2B and 2D, and the metabotropic receptor subunits mGluR1, 3, 4, 5, 6, 7 in at least 6 animals in two separate experiments, compared to normoxic controls (Figure 3). Again, no difference was observed between male and female pups.

Signaling pathway involved in the neuroprotective effect of iNO on excitotoxic-induced brain lesion

Finally, we explored a potential signaling pathway acting as a common modulator of glutamate-receptor expression in response to iNO. We hypothesized that CREB/Akt signaling pathway might be involved as CREB is recognized to bind the CRE sequence in the promoter of several glutamate receptor genes [18]. First, we found a significant reduction of pCREB protein concentration 24 h after the onset of NO exposure in P1 but not in P7 rat pups. This decrease was observed on ELISA assays, immunocytochemistry and western blot as well as (Figure 4A–D). To delineate upstream signaling pathways we further explore VEGF-Akt and ERK expression in response to NO exposure. We

found that iNO was associated with a significant decrease in brain VEGF concentration (ELISA) and gene expression (but no change in VEGFR1 and VEGFR2 using qPCR). Furthermore, iNO induced a decrease in Akt and phosphorylated Akt in P1 rat pups (Figure 4E). In contrast, Erk1/2 expression was found similar in iNO-exposed animals compared to controls (data not shown). In contrast to these early effects of iNO on pAkt/pCREB expression observed at P1, we found that iNO did not change pCREB protein level and induced a significant increased of pAkt protein concentration in P7 brains in response to excitotoxic challenge (Figure 4E). Therefore, we speculate that complex modulation of pAkt-pCREB signaling pathway by exogenous NO could be involved in its impact on glutamate receptors regulation and excitotoxic brain damage.

Discussion

We demonstrated here that iNO exposure during the first week of postnatal life significantly reduced the lesion size in an excitotoxic-induced brain lesions rat model. This effect appears to be associated with early downregulation of pCREB expression and subsequent downregulation of several glutamate receptors subunits.

It is now well established that NO is a physiological mediator of the central nervous system. The role of NO in developing brain remains poorly understood, but it seems to be involved in the

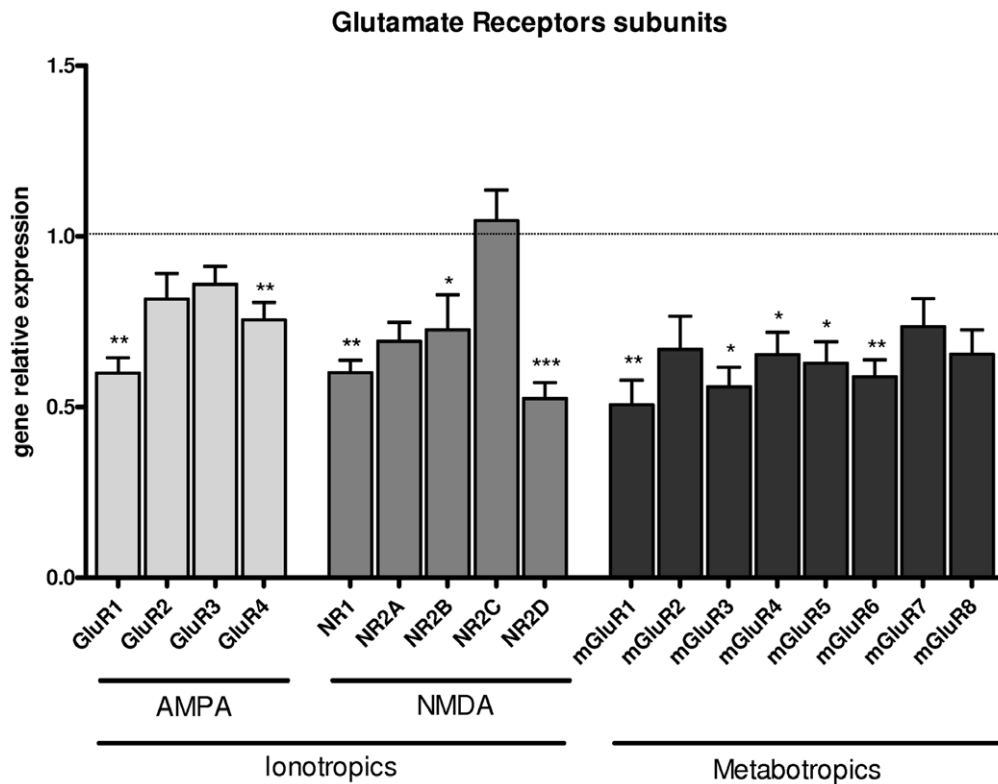


Figure 3. iNO downregulates several glutamate receptor subunits gene expression. Relative gene expression of glutamate receptor subunits in P5 rat pups subjected to 20 ppm iNO normalized to controls (*** <0.001 ; ** $p<0.01$; * $p<0.05$). doi:10.1371/journal.pone.0010916.g003

regulation of cerebral blood flow, and in memory acquisition. In fact, NO appears to be a double-edged sword, simultaneously neurotoxic and neuroprotective. Numerous experimental studies demonstrated the deleterious effects of nitrogen reactive species accumulation in ischemic-reperfusion cerebral injury through depletion of energy, lipid peroxidation, protein nitrosylation, DNA alterations and increased permeability of the blood brain-barrier [19–21]. Hypoxia-ischaemia results in inflammation, especially in the developing white matter. High concentrations of NO and peroxynitrite produced locally by activated microglia may become toxic to neurons and immature oligodendrocytes *in vitro* [22,23]. It is also well known that hypoxia-ischaemia results in the accumulation of extracellular glutamate, inducing the excitotoxicity cascade that causes neuronal death. More or less effective neuroprotection can be achieved by using NOS inhibitors that inhibit nNOS at the early phase and iNOS during the reperfusion of hypoxic insult [24].

In the other hand, endogenous NO could also result in contradictory effects, probably as a function of the intracellular redox state [25]. Endogenously produced NO in the brain also regulates local blood flow and could therefore offer neuroprotection [21,26]. An increase in brain infarct volume has been reported in the sheep and rat when NO production is decreased by NOS inhibitors [27]. Transgenic mice helped dissect out the respective contribution of nNOS, as the extent of the lesions after medial cerebral artery occlusion is reduced in eNOS^{-/-} [28], whereas secondary neuronal damage-induced by prolonged ischemia is lessened in iNOS^{-/-} [29]. The protection conferred on the ischemic brain by NO seems to be linked to vasodilatation which improves cerebral blood flow and hinders capillary microthrombi formation. Thus, NO seems to be beneficial on

the brain mostly through its vasodilatory effects, and maybe its potentially proangiogenic effects.

In contrast to the numerous studies focused on endogenous NO, almost no data was available on the experimental effect of exogenous inhaled NO. Extrapulmonary effect of iNO on reduction of myocardial infarction size and improved left ventricular systolic function have been shown in a murine model [30]. These studies demonstrate that an extrapulmonary vasoprotective effect of iNO is possible. There are several possible mechanisms for neuroprotection with iNO as well, such as modulation of circulating neutrophils, monocytes, and platelets as they pass through the lung [31]. iNO down-regulates lung-derived cytokines and free radicals production, which may lead to a decrease in brain injury [32]. Another possible mechanism in neuroprotection may relate to delivery of NO or NO-related metabolites, such as hemoglobin-derived S-nitrosothiol or nitrites/nitrates, which account for a distant vasodilatory activity [32,33]. NO may also play an important role in ischemic preconditioning *in vivo* [34].

Clinically, the impact of iNO on the development of the central system remains controversial. For many years, iNO was feared to increase the incidence of intracranial haemorrhage in critically ill preterm neonates, because NO was demonstrated to increase bleeding time and inhibit platelet aggregation [35]. Later clinical studies demonstrated there was no significant increase in intracranial bleeding in preterm neonates [31]. Furthermore, both Schreiber et al. [36], and Kinsella et al. [37] found a lower incidence of severe brain damage in the iNO treated group, respectively. More intriguing and exciting was the fact that this short-term improvement translated into a significant improvement in neurodevelopmental outcome in the group given iNO at two-

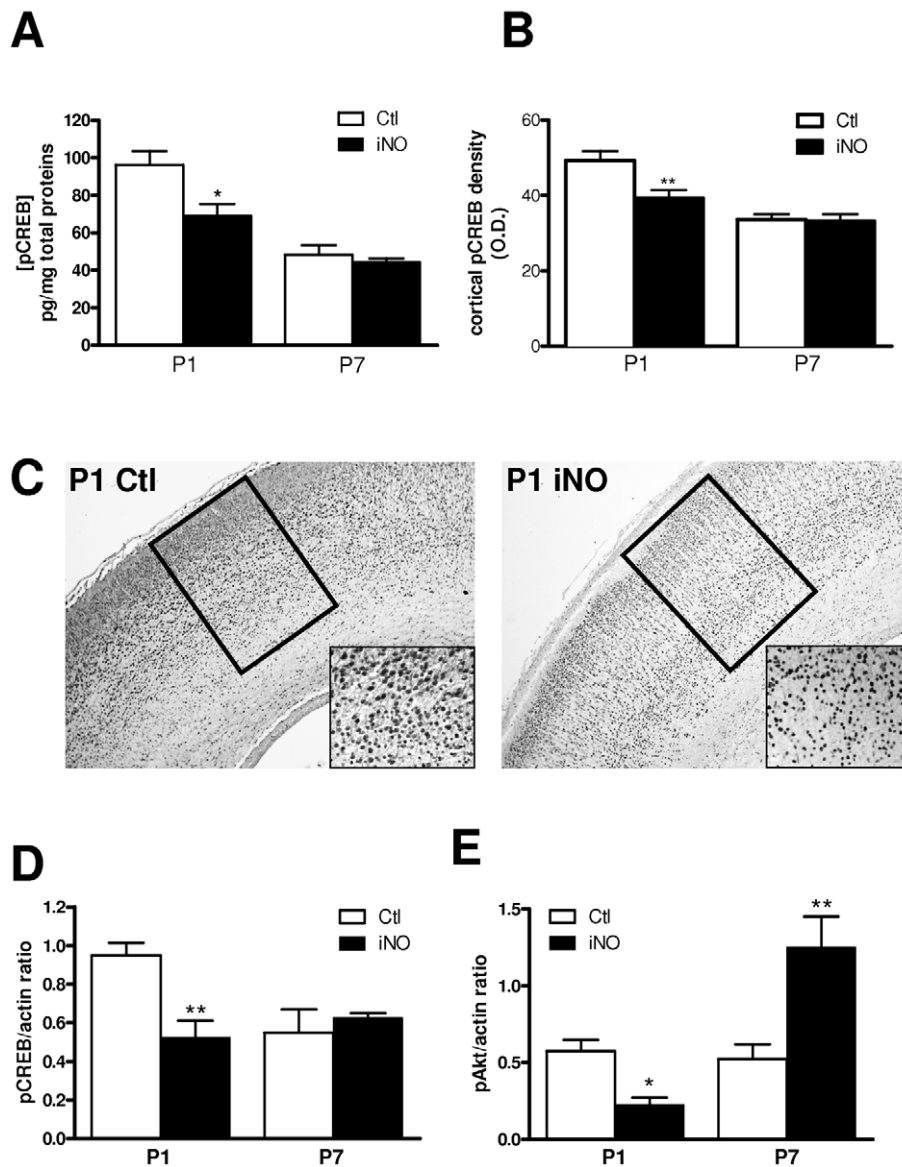


Figure 4. pAkt-pCREB pathway is involved in the neuroprotective effect of iNO. A. Quantitative analysis of pCREB protein content of P1 and P7 brain measured by ELISA assay (* $p < 0.05$). B. Quantitative analysis of cortical pCREB immunoreactivity optical density in P1 and P7 rat pups subjected to iNO compared to controls (** $p < 0.01$). C. Photomicrographs (with enlarged image) showing nuclear localization of cortical pCREB immunostaining in P1 rat pups with (iNO) or without (Ctl) NO (20 ppm) exposure. The area of optic density measurement (B) is indicated by a square. D, E. Quantitative analyses of western blot using pCREB (D) and pAkt (E) in response to iNO exposure (20 ppm) in P1 (before excitotoxic challenge) and P7 (after excitotoxic challenge) rat pups (* $p < 0.05$; ** $p < 0.01$). doi:10.1371/journal.pone.0010916.g004

year follow-up, and this was primarily due to a 47% decrease in the risk of cognitive impairment [38].

It is conceivable that a downregulation of glutamate receptors could reduce the lesion size induced by glutamate agonists. Here, we found that iNO-induced neuroprotection was associated with an early downregulation of pCREB expression and downregulation of several glutamate receptors subunits. CREB activates gene transcription in response to elevation of intracellular cAMP levels which in turn phosphorylates CREB at Ser133. Phosphorylated CREB binds to the cAMP response element (CREs), represented by the palindromic consensus sequence TGACGTC A found in the 5' flanking region of target genes [18]. This consensus sequence was found in the 5' flanking region of most of the iNO-regulated glutamate receptors genes (ie, NR1, NR2B, mGlu1, 3

and 6) but interestingly, was not found in the 5' flanking region of NR2C gene which transcription was unchanged in iNO-exposed rat pups (see Figure 2). Mutational analysis demonstrated that transcription of NR1 and NR2B are regulated by the c-AMP signaling pathway, most likely through the binding of CREB and its activation by signal-dependent phosphorylation [18,39]. Conversely, glutamate receptor activation and subsequent calcium influx may activate CREB shortly [40]. Moreover, most data indicated that pAkt was found neuroprotective [41]. In our model, CREB transcription level was found unchanged after the excitotoxic insult and pAkt protein level was significantly increased in response to excitotoxic challenge in P7 iNO-exposed rat pups as compared to room air-exposed controls. In addition to calmodulin-dependent kinases and MAPK/ERK kinases pathways, the

accumulation of cAMP in response to G-protein-coupled receptors also induces activation of Akt/protein kinase B which directly or indirectly affects CREB [42]. Here, our data suggest that exogenous NO induced subtle modulation of Akt/CREB signaling pathway in the developing brain. We speculate that iNO may induce an early downregulation of pCREB and subsequent upregulation of pAkt after excitotoxic insult leading to a decreased expression of several genes including those encoding for glutamate receptors subunits, and subsequent neuroprotection.

In conclusion, this study is the first to describe and to investigate the neuroprotective effect of iNO in neonatal excitotoxic-induced brain damage. This effect appears to be associated with changes in VEGF-pAkt-pCREB and glutamate receptor subunits expression. Further preclinical studies are needed to confirm the ability of iNO

to induce neuroprotection in other animal models of perinatal brain damage.

Supporting Information

File S1 Primary antibodies used for immunohistochemistry and western blot analyses.

Found at: doi:10.1371/journal.pone.0010916.s001 (0.04 MB DOC)

Author Contributions

Conceived and designed the experiments: JP GL PO RHF CCM JCM PG OB. Performed the experiments: JP GL OB. Analyzed the data: JP GL PO RHF CCM JCM PG OB. Contributed reagents/materials/analysis tools: JP OB. Wrote the paper: GL PG OB.

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Effet du NO inhalé sur le poumon et le cerveau en développement

Impact of inhaled NO on developing lung and brain

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Summary

With the advent of prenatal steroids, postnatal exogenous surfactant and less aggressive respiratory support, premature infants can develop chronic lung disease without even acute respiratory distress. This “new bronchopulmonary dysplasia” could be the result of impaired postnatal growth. Several experimental studies have suggested a possible role of the vascular endothelial growth factor/nitric oxide (VEGF/NO) pathway in restoring pulmonary angiogenesis and enhancing distal lung growth. The results of the clinical studies are, however, inconclusive, and it is currently unclear which subsets of premature infants might benefit from inhaled nitric oxide. Besides, severe intracranial haemorrhage and/or cystic periventricular leukomalacia may affect the most immature babies, many of whom are spared from severe initial respiratory disease. Recently, inhaled nitric oxide was shown to significantly decrease the incidence of these neurological events, and to improve the long-term outcome in a few clinical trials. At times neuroprotective, at times neurotoxic, nitric oxide is capable of divergent effects depending upon the extent of cerebral damage, the redox state of the cell, and the experimental model used. Recently, inhaled nitric oxide had recognized to have dramatic remote effects including angiogenesis and maturation on the developing brain in rodent pups. Therefore, the developmental consequences of inhaled NO should be further investigated to ensure its safety on the developing brain and to test its potential neuroprotective effect.

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Résumé

Depuis l'aire du surfactant exogène et des corticostéroïdes anténataux, les nouveau-nés prématurés évoluent souvent vers une nouvelle forme clinico-radiologique de dysplasie bronchopulmonaire parfois en l'absence de pathologie respiratoire initiale sévère. Cette nouvelle forme de pathologie pulmonaire chronique est principalement liée à la simplification capillaire et alvéolaire du poumon en développement. De nombreuses études expérimentales ont démontré l'implication de la voie de signalisation du VEGF/NO dans la restauration d'une angiogenèse et d'une alvéolisation normales. Les études cliniques testant l'administration de NO en période postnatale immédiate n'ont pu démontrer l'efficacité clinique de cette stratégie thérapeutique et il reste difficile d'isoler une sous-population de grands prématurés pouvant bénéficier du NO inhalé. Certains essais tendent à montrer que ce traitement pourrait avoir un impact positif sur l'évolution neurologique à moyen terme des enfants traités même si les résultats sont contradictoires d'une étude à l'autre. Expérimentalement, le NO peut avoir des propriétés neuroprotectrices ou neurotoxiques en fonction de l'état redox de la cellule et du protocole expérimental utilisé. Récemment, il a été démontré que le NO inhalé pouvait avoir un impact important sur l'angiogenèse et la maturation cérébrale remettant en cause le dogme de l'effet purement local de ce traitement. Ainsi, le NO inhalé devrait rapidement bénéficier d'études translationnelles approfondies afin de juger de son innocuité à long terme sur le plan neurodéveloppemental et d'étudier son potentiel neuroprotecteur.

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Mots-clés : NO, Dysplasie bronchopulmonaire, Développement cérébral.

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1. Introduction

Le monoxyde d'azote ou oxyde nitrique ou NO est un composé chimique formé d'un atome d'oxygène et d'un atome d'azote. C'est un gaz dans les conditions normales de pression et de température. C'est un important neurotransmetteur chez les mammifères et l'un des rares neurotransmetteurs gazeux connu.

Sa grande réactivité vis-à-vis des composés radicalaires comme l'oxygène ou l'anion superoxyde est due au fait qu'il est lui-même un composé radicalaire. En milieu biologique, sa demi-vie est estimée entre 1 et 5 secondes [1]. Les peroxy-nitrites obtenus sont de puissants agents oxydants et nitrants.

Le NO est une molécule endogène libérée notamment par les cellules endothéliales, les macrophages et les neurones. Il est synthétisé naturellement à partir de la L-arginine et de l'oxygène par plusieurs enzymes, les NO synthases (NOS), qui sont des hémoprotéines proches du cytochrome P450 (fig. 1). La liaison du NO aux thiols de bas poids moléculaire - tels que le glutathion - conduit à la formation de S-nitrosothiols qui constitueraient un déterminant important pour l'activité et le transport du NO. Ces composés peuvent agir comme donneurs de NO et ainsi augmenter substantiellement sa demi-vie à distance du site de production.

L'activité cellulaire du monoxyde d'azote passe par deux voies essentielles, celle qui consiste en la production de Guanosine monophosphate cyclique intra-cellulaire (GMPC) à partir de la Guanosine triphosphate (GTP), et la formation de peroxy-nitrites cytotoxiques. À lui seul, le GMPC est responsable d'un grand nombre d'effets biologiques du NO résumés dans la figure 2.

Dans l'organisme le monoxyde d'azote remplit plusieurs fonctions :

- Effet vasodilatateur. Le NO a la capacité de déclencher le relâchement de la couche musculaire lisse vasculaire, provoquant ainsi une vasodilatation et un accroissement du débit sanguin et une diminution de l'agrégation des plaquettes sanguines [2].

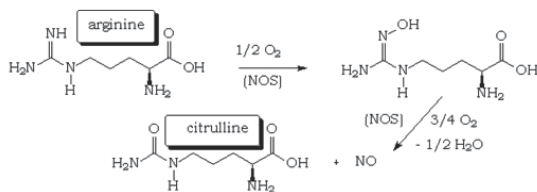


Figure 1 : Schéma de synthèse du NO.

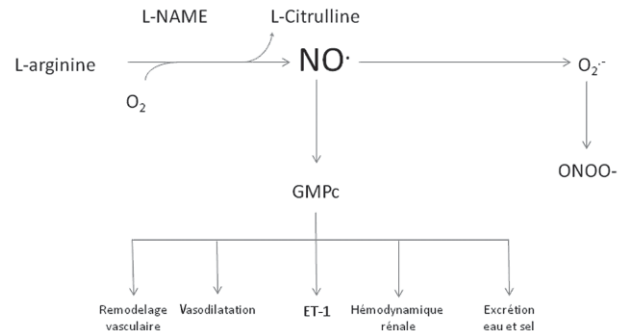


Figure 2 : Effets biologiques du GMPC.

- Microbicide. Les macrophages produisent du NO dans le but d'éliminer des bactéries pathogènes, NO qui peut, dans certaines circonstances, provoquer des effets secondaires nuisibles : c'est le cas dans les infections fulminantes où la production excessive de NO par les macrophages conduit à une vaso-dilatation massive, cause principale de l'hypotension rencontrée dans le choc septique.
- Neurotransmetteur. Le NO joue le rôle d'un neurotransmetteur [3]. À la différence de la majorité des autres neurotransmetteurs, dont l'action dans la fente synaptique a pour cible unique le neurone post-synaptique, la molécule de NO diffuse largement et peut atteindre plusieurs neurones environnants, y compris des neurones non-inter connectés par des synapses. Ce processus serait impliqué dans la mémorisation en assurant la mémorisation à long terme [4].
- Relaxation des muscles lisses extravasculaires. Le NO est présent dans plusieurs organes du tube digestif comme neurotransmetteur non-adrénergique, non-cholinergique [5]. Il est responsable de la relaxation des muscles lisses de l'appareil gastro-intestinal.
- Régulateur de l'apoptose. Le monoxyde d'azote est un régulateur essentiel de l'apoptose cellulaire. Il peut avoir un effet anti-apoptotique, ou, inversement, un effet apoptotique [6]. Cette bascule est intimement liée à la présence ou non de réducteurs cellulaires. En présence d'un taux élevé d'ion superoxyde O₂⁻, le monoxyde d'azote permet la formation d'ion peroxy-nitrite, probablement responsable de la modification du potentiel de membrane mitochondrial ainsi que de nombreux phénomènes d'apoptose cellulaire, en particulier celle des cellules du système immunitaire.

2. NO et poumon au cours du développement

Le NO a un rôle fondamental d'une part dans le contrôle du tonus vasculaire pulmonaire et d'autre part dans l'organogénèse et l'angiogénèse pulmonaire. Deux pathologies néonatales bien connues illustrent ces deux propriétés : l'hypertension artérielle persistante du nouveau-né (HTAPPNN) et la dysplasie broncho-pulmonaire (DBP) du prématuré.

2.1. NO et HTAPPNN

Le NO synthétisé par les cellules endothéliales joue un rôle clé dans le contrôle du tonus et de la trophicité des artères pulmonaires. L'HTAP, qui associe augmentation du tonus vasomoteur et importants remaniements structuraux des artères pulmonaires, s'accompagne d'une inadaptation de la formation de NO. L'inhibition de la formation de NO dans la circulation pulmonaire fœtale reproduit les anomalies hémodynamiques caractéristiques de l'HTAP du nouveau-né et l'apport de NO exogène protège contre le développement de l'HTAP chez l'animal exposé à l'hypoxie [7]. La circulation pulmonaire est en effet facilement accessible au NO exogène délivré par inhalation qui relaxe le muscle lisse des artérioles pulmonaires contiguës aux alvéoles respiratoires. Cette propriété du NO inhalé est à la base de son utilisation thérapeutique dans l'HTAPPNN.

Cinq pour cent des détresses respiratoires néonatales se compliquent d'hypoxie sévère ou réfractaire. Cette hypoxie sévère est liée soit à un shunt intrapulmonaire, soit à un shunt extrapulmonaire droite-gauche, du fait d'une augmentation des résistances vasculaires pulmonaires (RVP), réalisant l'HTAPPNN [8]. La cellule endothéliale vasculaire possède un rôle central dans la régulation de la vasomotricité, car elle libère de nombreux médiateurs locaux dont le NO qui joue un rôle essentiel dans la vasodilatation.

Le traitement de l'HTAPPNN repose sur la correction des anomalies métaboliques et hématologiques, le maintien d'une pression artérielle systémique correcte et l'utilisation de vasodilatateurs. Les vasodilatateurs systémiques tels que la tolazoline ou la prostaglandine I₂ ont été utilisés, mais leurs effets sur la pression artérielle systémique expliquent l'irrégularité de leur efficacité [9]. Le NO est le traitement actuel le plus efficace. Le NO est lipophile et diffuse volontiers au muscle lisse adjacent. Il se lie alors avec la guanylate-cyclase soluble pour donner de la GMPc à partir de la GMP. La GMPc produit son action vasodilatatrice en entraînant la baisse du calcium cytosolique. La GMPc est dégradée en composant inactif sous l'action des phosphodiésterases. La demi-vie du

NO est très courte de l'ordre de 3 à 6 secondes. Sa liaison avec l'hémoglobine entraîne la formation de nitrosylhémoglobine, qui est à l'origine de la formation de méthémoglobine. La rapidité de son métabolisme, explique l'absence d'effet systémique apparent du NO inhalé [8]. Par ailleurs, le NO inhalé peut réguler négativement la NOS endothéliale, ce qui explique que son sevrage doit être progressif pour éviter tout rebond hypertensif pulmonaire. Le NO a été largement étudié ces dernières années en pédiatrie. L'amélioration de l'oxygénation est observée dans de nombreuses séries [10-14]. En revanche, il n'est pas observé de diminution de la mortalité par rapport au groupe témoin. En cas de pathologie parenchymateuse, la faible expansion pulmonaire peut être à l'origine d'un échec du NO, d'où la nécessité de faire une stratégie de recrutement alvéolaire [10]. Dans cet objectif, on peut utiliser la PEEP, le surfactant exogène, la ventilation à haute fréquence ou la ventilation liquide [10]. Dans une étude récente, le surfactant s'est néanmoins révélé moins efficace dans cette indication pour diminuer le recours à l'ECMO [15] qui reste la thérapeutique ultime de sauvetage [16].

2.2. NO et DBP

L'efficacité, au moins à court terme, du traitement par NO inhalé chez les nouveau-nés à terme présentant une insuffisance respiratoire hypoxique a été prouvée. La physiopathologie de l'insuffisance respiratoire, et les risques potentiels, sont substantiellement différents chez les nouveau-nés prématurés. Par conséquent, l'analyse de l'efficacité et de la toxicité du monoxyde d'azote inhalé chez les nouveau-nés nés avant 35 semaines d'âge gestationnel est nécessaire.

2.2.1. Études expérimentales

Plusieurs études expérimentales ont démontré le rôle critique de l'inhalation de NO sur l'angiogénèse et alvéolarisation pulmonaire, deux phénomènes critiques dans la constitution des lésions de DBP. Chez la souris nouveau-née invalidée pour le gène codant pour eNOS, une hypoxie de 10 jours entraîne une réduction du nombre d'alvéoles associée à une diminution de la densité vasculaire et à une diminution du poumon en VEGFR-2 [17]. Pour déterminer si l'angiogénèse est nécessaire pour une alvéolarisation normale, les effets de deux anti-angiogéniques, thalidomine et fumagilline, ont été étudiés au cours d'une période critique de la croissance des poumons chez les rats nouveau-nés [18]. Ces médicaments réduisent le poids du poumon et la densité artérielle pulmonaire de 20 et 36 %, respectivement. Même si ces molécules peuvent avoir des effets non spécifiques de la croissance du poumon, le SU-

5416, inhibiteur spécifique de VEGFR-2 (KDR/flk-1) diminue également l'alvéolarisation et la densité artérielle suggérant que des agents anti-angiogéniques réduisent la croissance et l'alvéolarisation pulmonaires néonatales chez le rat.

L'inhibition des récepteurs du VEGF chez le rat nouveau-né diminue la croissance vasculaire et l'alvéolarisation pulmonaire et provoque une HTAP ; ainsi, les effets de l'inhalation de NO, un médiateur de l'effet du VEGF, ont été étudiés dans des rats nouveau-nés qui ont reçu une dose unique de SU-5416 [7]. Les rats nouveau-nés traités présentent une diminution de l'expression pulmonaire de eNOS ; l'inhalation de NO à 10 ppm diminue l'HTAP induite par le SU-5416 et améliore la croissance pulmonaire.

Dans un modèle de babouins atteints de maladie pulmonaire chronique de type DBP, 5 ppm de NO inhalé pendant 14 jours induisent une diminution de la pression artérielle pulmonaire, et un taux plus élevé de fermeture spontanée du canal artériel [19]. En outre, l'excès de dépôts d'élastine caractéristique de la DBP a été normalisé par l'inhalation de NO.

2.2.2. Études cliniques

Une méta-analyse récente de l'effet du traitement par monoxyde d'azote sur les taux de mortalité, de dysplasie bronchopulmonaire (DBP), d'hémorragie intraventriculaire, ou d'anomalie du développement neurologique chez les nouveau-nés prématurés (< 35 semaines d'âge gestationnel) présentant une maladie respiratoire a été réalisée [20]. Onze essais comparatifs randomisés sur le traitement par NO inhalé chez les nouveau-nés prématurés ont été identifiés. Les essais ont été regroupés en trois catégories en fonction des critères d'inclusion ; l'inclusion au cours des trois premiers jours de vie se basait sur les critères d'oxygénation, l'utilisation systématique chez les nouveau-nés prématurés intubés et l'inclusion tardive basée sur un risque accru de DBP [20 pour revue]. Les essais de traitement curatif basés sur les critères d'oxygénation n'ont démontré aucun effet significatif du NO inhalé sur la mortalité ou la DBP. Le sous-groupe d'études avec une utilisation préventive systématique du NO inhalé chez les prématurés intubés est associé à une légère réduction significative du résultat combiné de décès ou de DBP (RR 0,91 (95 % CI 0,84, 0,99)). L'administration de NO inhalé curative chez des prématurés à haut risque de DBP ultérieure n'a démontré aucun avantage. Les études curatives précoces sont associées à une tendance à l'augmentation d'hémorragies intraventriculaires sévères, alors que le sous-groupe d'études avec une utilisation préventive chez les prématurés intubés semble montrer une réduction de ce risque. Deux études [21, 22] rapportent

les données sur le développement neurologique à 2 ans des enfants exposés. Un traitement préventif semble être bénéfique alors qu'aucune différence n'est notée en cas de traitement curatif.

Ainsi, l'utilisation du NO comme traitement de « rattrapage » chez les nouveau-nés prématurés très malades sous ventilation mécanique ne semble pas être efficace et pourrait augmenter le risque d'hémorragie intraventriculaire grave. L'utilisation tardive du monoxyde d'azote dans la prévention de la DBP ne semble pas non plus être efficace. L'utilisation systématique de monoxyde d'azote chez les nouveau-nés prématurés plus légèrement malades pourrait réduire le risque de lésions cérébrales et améliorer le taux de survie sans DBP. De nouveaux essais (notamment EUNO) doivent permettre de conclure très prochainement et de définir les groupes les plus susceptibles de bénéficier du traitement. Un suivi à long terme des cohortes de ces essais devra également prouver l'innocuité du NO inhalé notamment sur le plan neurodéveloppemental.

3. NO et cerveau en développement

Malgré l'amélioration de la survie d'un nombre important de très grands prématurés nés à moins de 28 semaines d'aménorrhée, l'incidence de l'infirmité motrice cérébrale (IMC), évaluée à environ 2 pour 1 000 enfants nés vivants, est restée constante ces vingt dernières années [23]. L'incidence des lésions cérébrales atteint 7 à 26 % des prématurés dont le poids de naissance est inférieur à 1 500 g et 60 à 100 % des enfants présentant des lésions de leucomalacie périventriculaire cavitaire développent un handicap. Il n'y a actuellement aucun traitement prophylactique ou curatif des lésions cérébrales du nouveau-né. La recherche de nouvelles stratégies de neuroprotection est un besoin crucial de santé publique.

Parmi les facteurs de risque majeurs de lésions cérébrales périnatales chez le prématuré figure l'accumulation de radicaux libres, qu'ils soient dérivés de l'oxygène ou du NO [23, 24]. La production de NO endogène est reconnue comme jouant un rôle clé dans l'organogenèse chez les vertébrés notamment au niveau pulmonaire et cérébral. Certaines observations cliniques suggèrent que le NO exogène pourrait avoir un rôle protecteur sur les systèmes respiratoire et cardiovasculaire pendant une période critique du développement chez le prématuré. Cependant, très peu de données sont disponibles sur les effets du NO inhalé potentiellement bénéfiques sur le cerveau en développement et son impact en cas d'agression hypoxo-ischémique périnatale.

Le NO inhalé est actuellement une des thérapeutiques néonatales les plus controversées, proposées à un grand nombre de nouveau-nés prématurés bien que très peu de données soient disponibles sur ses effets sur le cerveau en développement et son impact en cas d'agression hypoxo-ischémique.

3.1. Stress oxydatif et vulnérabilité du cerveau en développement

Dans les conditions physiologiques, un équilibre existe entre la quantité de radicaux libres dérivés de l'oxygène ou du NO (ROS/RNS) produits par le métabolisme cellulaire normal et le système de défenses anti-oxydantes endogènes. À la naissance, le nouveau-né est exposé à une PaO₂ artérielle 5 fois plus élevée par rapport à celle du fœtus [25]. D'autre part, chez le nouveau-né prématuré, l'infection est une cause importante de travail prématuré [26] et l'activation des leucocytes polynucléaires périnataux associés à l'inflammation peut accroître le stress oxydatif en générant des ROS et RNS après l'activation des NOS.

En même temps, les enzymes antioxydantes et les mécanismes de défenses contre les radicaux libres ne sont pas complètement développés chez les prématurés [27]. La substance blanche immature des prématurés est peuplée d'oligodendrocytes immatures qui sont particulièrement sensibles aux stress excitotoxique et oxydatif [28, 29]. Des études *in vitro* ont montré que la vulnérabilité des oligodendrocytes aux ROS et RNS variait au cours de leur maturation [29-32]. Ainsi, la substance blanche immature du prématuré est très vulnérable à l'exposition périnatale d'une inflammation et/ou d'un stress oxydatif survenant en période périnatale.

3.2. Le NO : neuroprotecteur ou neuro-aggravant ?

L'impact du NO inhalé sur le développement du système central du prématuré reste controversé. Pendant de nombreuses années, le NO inhalé a été considéré comme responsable d'une augmentation de la fréquence des hémorragies intracrâniennes chez le nouveau-né prématuré, parce qu'expérimentalement, il avait été démontré que le NO augmente le temps de saignement et inhibe l'agrégation plaquettaire. Des études cliniques plus récentes démontrent qu'aucune augmentation significative des hémorragies intracrâniennes chez les prématurés (peu ou pas malades sur le plan respiratoire) n'a été rapportée [33]. Par ailleurs, les études de Schreiber et al. [22], et de Kinsella et al. [34] ont montré que l'administration de NO inhalé était associée à une incidence plus faible d'hémorragies intraventriculaires de haut grade et de leucomalacie périventriculaire. Plus intéressant est le fait que cette amélioration à

court terme se traduit par une amélioration significative des évolutions neurodéveloppementales dans le groupe traité par le NO inhalé à deux ans avec une diminution de 47 % du risque de troubles cognitifs [35].

Il est maintenant bien établi que le NO est un médiateur physiologique indispensable au développement du système nerveux central [36]. Au sein du parenchyme cérébral, le NO est produit par les NO synthases exprimées au niveau neuronal (nNOS), glial (iNOS, exprimée par les astrocytes, la microglie, et les oligodendrocytes) et endothéliale (eNOS). Malgré un certain degré de spécificité, chaque type cellulaire est capable d'exprimer toutes les isoformes de NOS. Le rôle du NO dans le cerveau en développement reste mal compris, mais il semble être impliqué notamment dans la régulation du débit sanguin cérébral, et dans le développement de la mémoire.

En cas d'atteinte cérébrale, l'activité NOS peut être augmentée, produisant localement en excès du NO. Ce phénomène s'observe en cas de stimulation excessive des récepteurs du glutamate ou par excès de libération de cytokines [37]. En cas d'ischémie-reperfusion, il existe une nette augmentation du NO endogène produit au cours de la phase de reperfusion et/ou réoxygénation, augmentation qui est corrélée avec l'intensité de l'atteinte hypoxo-ischémique [38]. Cette variation de la production de NO dépend de la région du cerveau considérée : elle est surtout observée dans le cortex, l'hippocampe, l'hypothalamus, l'amygdale, et la substantia nigra [39]. Les lésions histologiques de l'hippocampe induites par occlusion de l'artère cérébrale moyenne chez le rat ont été corrélées à la concentration tissulaire de NO et de ses dérivés [40].

En fait, le NO semble être une molécule dont l'action est ambivalente, à la fois neurotoxique et neuroprotectrice. De nombreuses études expérimentales ont démontré les effets néfastes de l'accumulation de NO dans l'atteinte cérébrale par ischémie-reperfusion [41]. Les mécanismes moléculaires par lesquels l'excès de NO conduit à la mort neuronale impliquent la défaillance énergétique, la peroxydation des lipides, la nitrosylation des protéines et l'atteinte structurelle de l'ADN. En cas d'inflammation de la substance blanche, des concentrations élevées de NO et de peroxyde d'azote produit localement par la microglie activée deviennent toxiques pour les neurones et les oligodendrocytes immatures *in vitro* [42]. L'administration d'inhibiteurs des NOS induit un effet neuroprotecteur plus ou moins efficace, notamment au cours de la phase précoce (rôle de nNOS) et de la phase de reperfusion (rôle de iNOS) [43].

Toutefois, de nombreuses études ont montré que l'inhibition des NOS aboutit à des effets contradictoires, probablement en raison de la variation de l'état redox intracellulaire [44]. L'effet délétère de l'excès de NO sur la survie des cellules

semble être lié en partie à son action sur les mitochondries (effet inhibiteur sur la chaîne respiratoire, induction de la perméabilité membranaire et libération intracellulaire du cytochrome C et de protéines pro-apoptotiques) [32].

À l'inverse, une augmentation de volume de l'infarctus cérébral a été rapportée chez le mouton et le rat lorsque la production de NO est pharmacologiquement inhibée [45]. La protection conférée par le NO envers une atteinte hypoxo-ischémique semble être liée à l'adaptation vasculaire au cours de la reperfusion, augmentant le débit sanguin cérébral et empêchant la formation de microthrombi capillaires [46]. Ainsi, le NO semble être bénéfique sur le cerveau le plus souvent par le biais de ses effets vasodilatateurs, et peut-être par ses effets potentiellement pro-angiogéniques.

3.3. Effets expérimentaux du NO inhalé sur le développement cérébral

L'effet du NO inhalé n'a jamais été étudié expérimentalement au cours du développement cérébral normal. Des études très récentes ont démontré expérimentalement que le NO pouvait avoir un impact non seulement sur le poumon, localement, mais aussi sur le foie et le cerveau, à distance [46]. Le (s) médiateur (s) sérique (s) de ces effets (NO, nitrosothiols, nitrites, nitrates...) reste (nt) encore inconnu (s). Cependant, le dogme de l'effet purement local du NO inhalé semble incorrect. Dans un protocole expérimental testant le NO inhalé (5 et 20 ppm) administré pendant 3 ou 7 jours en postnatal, les rats exposés présentent une augmentation significative de la quantité de nitrites dans le sérum démontrant une

diffusion systémique du NO exogène [47]. Les rats soumis à 5 ou 20 ppm de NO présentent une augmentation de la densité vasculaire de plus de 40 % par rapport aux contrôles indépendamment de la production endogène de NO (fig. 3). Ainsi, il existe à des concentrations proches de celles employées chez le grand prématuré un effet trophique du NO inhalé sur l'angiogenèse et la croissance somatique. L'effet du NO inhalé sur l'angiogenèse semble emprunter des voies différentes selon qu'elle intervienne sur un poumon ou un cerveau en développement : la voie du VEGF dans le premier cas et la modification de la balance entre facteurs pro- et anti-angiogéniques dans le second.

Le NO inhalé apparaît comme un puissant modulateur des processus de myélinisation chez le rat en conditions normales. En effet, des animaux exposés au NO présentent une augmentation de la maturation oligodendrocytaire associée à une myélinisation accrue de la substance blanche au cours du développement. Des études comportementales ne montrent aucun effet délétère de ce phénomène chez l'animal sain. Au contraire, les animaux traités en période néonatale par un inhibiteur des NOS ont des performances cognitives à l'âge adulte bien inférieures à celles des animaux contrôles. Ce déficit est corrigé par l'administration de NO inhalé durant la période néonatale, prouvant que la présence de NO est indispensable au développement cérébral normal.

Ces différents effets cérébraux du NO inhalé, nouvellement décrits, doivent servir de base pour :

- mieux comprendre comment le NO inhalé module directement ou via des médiateurs intermédiaires la formation de myéline dans le cerveau en développement ;

Contrôle



NO inhalé 5 ppm

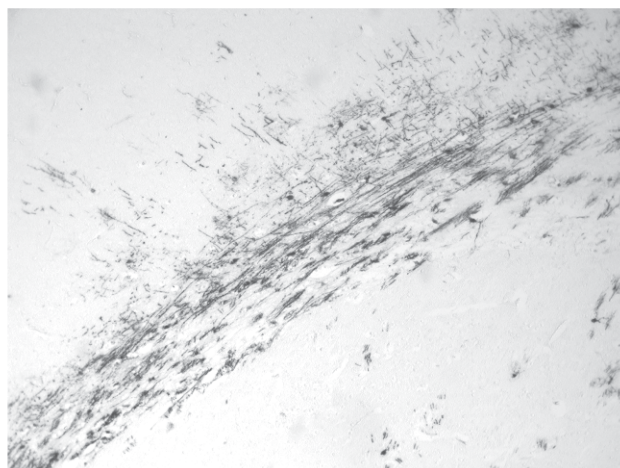


Figure 3 : Augmentation du contenu en myéline (MBP) chez le raton P7 soumis à 5 ppm de NO inhalé en période postnatale.

- connaître le potentiel neuroprotecteur du NO exogène sur les lésions de la substance blanche et le défaut de myélinisation qui en découle.

L'effet du NO inhalé sur la myélinisation et son potentiel neuroprotecteur sont deux découvertes importantes qui nécessitent d'être explorées :

- afin d'en découvrir les mécanismes moléculaires impliqués dans l'accélération de la maturation oligodendrocytaire sont intéressants sur un plan fondamental ;
- l'administration de NO inhalé est actuellement très débattue dans les soins aux grands prématurés et il est nécessaire d'en connaître davantage sur son impact sur le cerveau en développement ;
- afin de mieux comprendre le potentiel neuroprotecteur du NO inhalé qui pourrait représenter une nouvelle stratégie de prévention des lésions du cerveau immature et/ou de promouvoir la réparation d'un cerveau déjà lésé ;
- l'effet neuroprotecteur déjà observé, s'il se généralise dans d'autres modèles de lésions cérébrales périnatales, pourrait être testé sur des modèles de pathologies adultes.

4. Conclusion

Le NO est largement utilisé en néonatalogie avec des effets à court terme souvent spectaculaires mais peu d'effets significatifs à plus long terme, tant sur le plan curatif (HTAP, DBP) que préventif des formes sévères de DBP. L'impact de l'administration de NO inhalé sur le développement pulmonaire et cérébral est multiple touchant l'angiogenèse, la trophicité et la maturation de ces deux organes. Le dogme de l'effet exclusivement locorégional du NO sur le poumon semble faux tant ce gaz est expérimentalement associé à des conséquences maturatives importantes sur le cerveau en développement. Ainsi, il pourrait s'avérer que de nouvelles indications thérapeutiques soient testées cliniquement notamment sur le plan de la neuroprotection.

Conflits d'intérêts : O. Baud et P. Olivier : Essais cliniques : en qualité d'investigateur principal, coordinateur ou expérimentateur principal (Ikaria) ; Versements substantiels au budget d'une institution - «Research grant» (Ikaria).

G. Vottier, H. Pham, JC Mercier et G. Loron : aucun.

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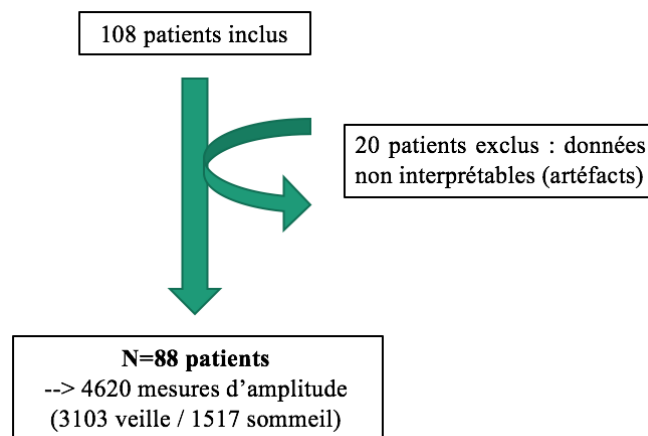
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NORMES aEEG de 1 mois à 18 ans

Résultats du travail de thèse du Dr Margaux Descharmes

Flow chart et population

Il y a eu 108 patients inclus dans cette étude ; 20 patients ont été secondairement exclus car les données d'aEEG étaient ininterprétables car trop artéfactées. Au total, les données de 88 patients ont été prises en compte.



Nombre	
1mois-3mois	9
3mois-6mois	8
6mois-1an	12
1an-3ans	16
3ans-6ans	11
6ans-12ans	22
13ans-18ans	10
TOTAL	88

TABEAU I : Normes aEEG 1 mois-3 mois

1 MOIS-3 MOIS	VEILLE		SOMMEIL	
	marge supérieure	marge inférieure	marge supérieure	marge inférieure
Fp1-C3	49,45 (36,26-57,31)	11,20 (8,27-11,97)	77,86 (62,16-84,17)	13,66 (11,91-16,18)
Fp2-C4	48,29 (33,72-54,74)	10,33 (7,89-11,12)	77,86 (62,16-80,90)	13,42 (11,91-14,92)
Fp1-T3	40,35 (35,66-50,28)	8,80 (8,11-10,11)	54,22 (53,49-57,66)	12,16 (11,33-12,84)
Fp2-T4	43,91 (33,83-51,29)	9,13 (7,47-10,29)	53,49 (51,56-56,89)	11,20 (10,25-12,09)
C3-O1	55,85 (52,42-65,91)	10,83 (9,76-12,84)	82,60 (76,13-83,93)	16,09 (14,92-17,38)
C4-O2	52,76 (45,83-59,97)	10,68 (9,74-11,99)	77,86 (62,50-83,93)	16,09 (14,92-17,34)
T3-O1	47,02 (39,70-54,90)	9,63 (8,74-11,03)	62,16 (51,17-72,23)	12,11 (11,55-13,85)
T4-O2	46,21 (44,91-50,40)	9,89 (9,38-11,99)	62,16 (56,26-67)	13,78 (13,18-13,85)
C3-C4	39,78 (30,27-49,44)	8,74 (6,64-9,91)	62,16 (53,49-74,24)	11,95 (11,81-14,92)

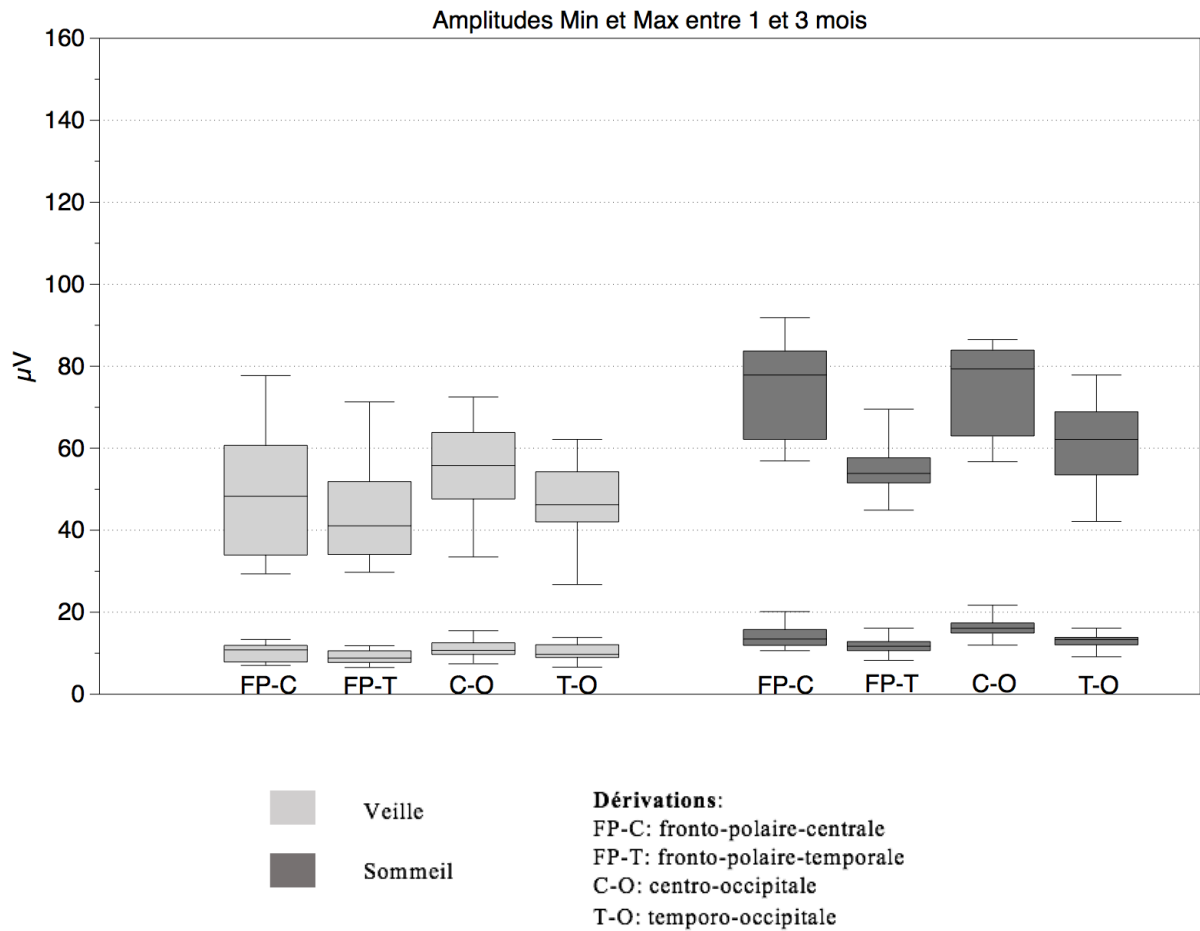


TABLEAU II : Normes aEEG 3 mois-6 mois

3 MOIS-6 MOIS	VEILLE		SOMMEIL	
	marge supérieure	marge inférieure	marge supérieure	marge inférieure
Fp1-C3	64,58 (61,84-73,38)	11,97 (11,49-12,60)	90,47 (85,87-97,53)	16,09 (13,62-17)
Fp2-C4	68,92 (62,41-73,01)	14,36 (12,28-15,49)	90,47 (67,33-97,53)	17,34 (13,62-18,70)
Fp1-T3	77,86 (70,09-81,91)	13,24 (11,36-16,09)	77,86 (65,23-83,93)	14,92 (10,00-16,09)
Fp2-T4	64,58 (61,11-68,74)	13,25 (12,16-14,21)	72,23 (64,72-77,86)	13,76 (13,76-16,09)
C3-O1	59,66 (54,13-71,23)	13,88 (12,30-16,09)	90,47(83,99-90,47)	20,15 (15,47-20,35)
C4-O2	66,43 (52,52-83,93)	13,58 (11,96-16,62)	86,90 (83,93-86,90)	18,70 (14,82-21,72)
T3-O1	62,61 (61,18-83,93)	12,95 (12,74-15,53)	83,93 (80,17-83,93)	18,70 (16,83-18,91)
T4-O2	67,10 (61,27-73,90)	14,15 (12,37-16,09)	79,88 (79,88-83,93)	16,18 (16,18-17,34)
C3-C4	54,85 (50,38-67)	11,76 (10,93-13,77)	73,69 (73,69-90,47)	16,09 (15,47-16,42)

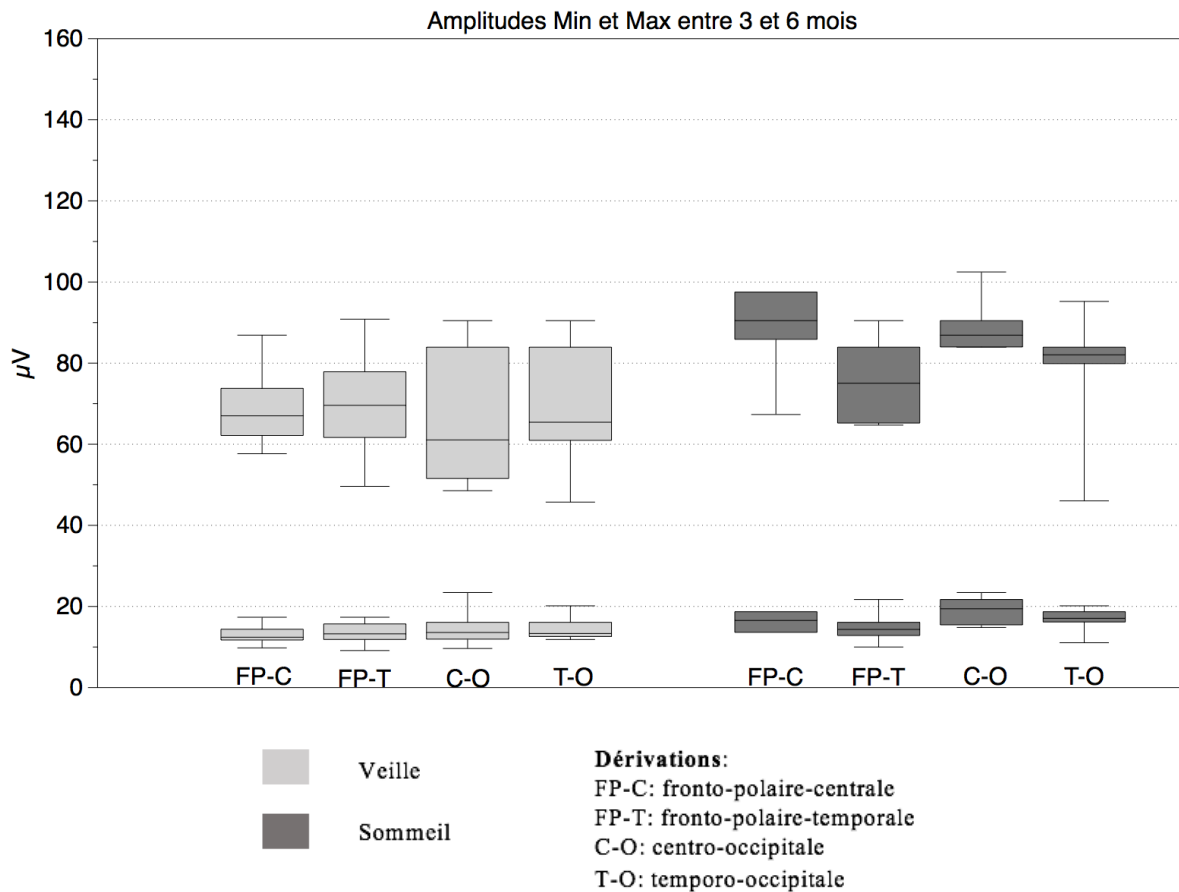


TABLEAU III : Normes aEEG 6 mois-1 an

6 MOIS-1 AN	VEILLE		SOMMEIL	
	marge supérieure	marge inférieure	marge supérieure	marge inférieure
Fp1-C3	64,44 (59,64-70,51)	12,83 (11,26-14,08)	86,90 (85,35-86,90)	16,85 (15,35-17,73)
Fp2-C4	70,39 (64,18-72,49)	13,62 (11,81-15,27)	90,01 (86,95-94,13)	18,04 (17,62-18,89)
Fp1-T3	66,13 (59,75-72,76)	13,67 (12,68-14,80)	86,90 (84,83-86,90)	17,78 (16,59-20,75)
Fp2-T4	72,18 (69,78-81,83)	13,93 (13,27-16,15)	90,78 (88,71-93,93)	18,91 (16,51-20,91)
C3-O1	77,73 (74,29-81,41)	17,53 (15,75-18,62)	93,33 (90,81-94,71)	20,86 (20,29-21,38)
C4-O2	79,29 (76,94-82,20)	17,25 (14,21-17,75)	88,47 (86,90-92,11)	20,75 (19,31-22,58)
T3-O1	69,64 (66,15-75,39)	15,31 (14,16-17,73)	90,29 (87,20-92,72)	17,82 (16,27-20,13)
T4-O2	92,11 (86,90-94,88)	20,96 (20,19-22,66)	92,11 (86,90-94,88)	20,96 (20,19-22,66)
C3-C4	54,37 (52,52-59,38)	12,14 (11,20-12,63)	88,47 (82,22-90,72)	18,91 (16,12-19,08)

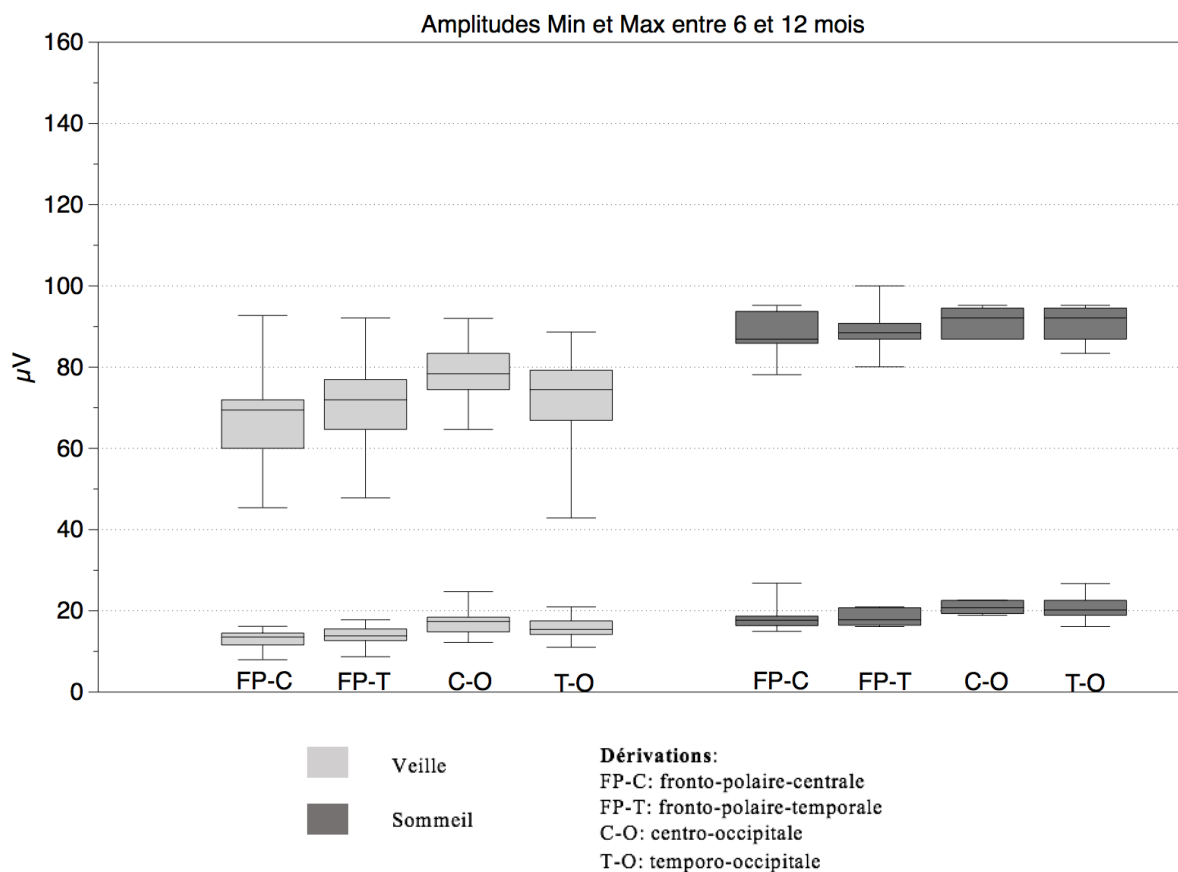


TABLEAU IV : Normes aEEG 1 an-3 ans

1 AN-3 ANS	VEILLE		SOMMEIL	
	marge supérieure	marge inférieure	marge supérieure	marge inférieure
Fp1-C3	67,66 (65,31-76,38)	15,75 (13,04-16,60)	84,39 (83,30-88,02)	15,17 (14,19-16,45)
Fp2-C4	69,42 (65,94-79,02)	15,75 (13,43-17,62)	84,39 (82,12-98,83)	15,85 (15,10-20,56)
Fp1-T3	71,14 (63,88-77,84)	15,36 (13,88-16,64)	86,43 (82,96-95,22)	17,69 (16,30-19,48)
Fp2-T4	76,38 (70,99-84,92)	16,68 (15,32-18,60)	90,76 (84,95-96,42)	18,33 (16,38-21,96)
C3-O1	73,68 (70,97-80,41)	17,05 (15,92-18,25)	84,82 (78,25-91,88)	17,42 (15,74-19,11)
C4-O2	76,38 (72,94-80,17)	16,86 (15,43-18,52)	94,70 (92,80-97,04)	17,24 (16,81-18,60)
T3-O1	74,07 (67,06-82,20)	16,63 (15,45-18,29)	90,16 (83,19-96,17)	17,59 (16,31-19,19)
T4-O2	76,38 (70,22-86,90)	16,32 (15,22-19,27)	96,42 (92,69-97,80)	18,87 (18,46-24,51)
C3-C4	57,98 (48,79-72,33)	12,95 (11,55-14,45)	79,31 (70,76-92,99)	15,37 (13,59-17,98)

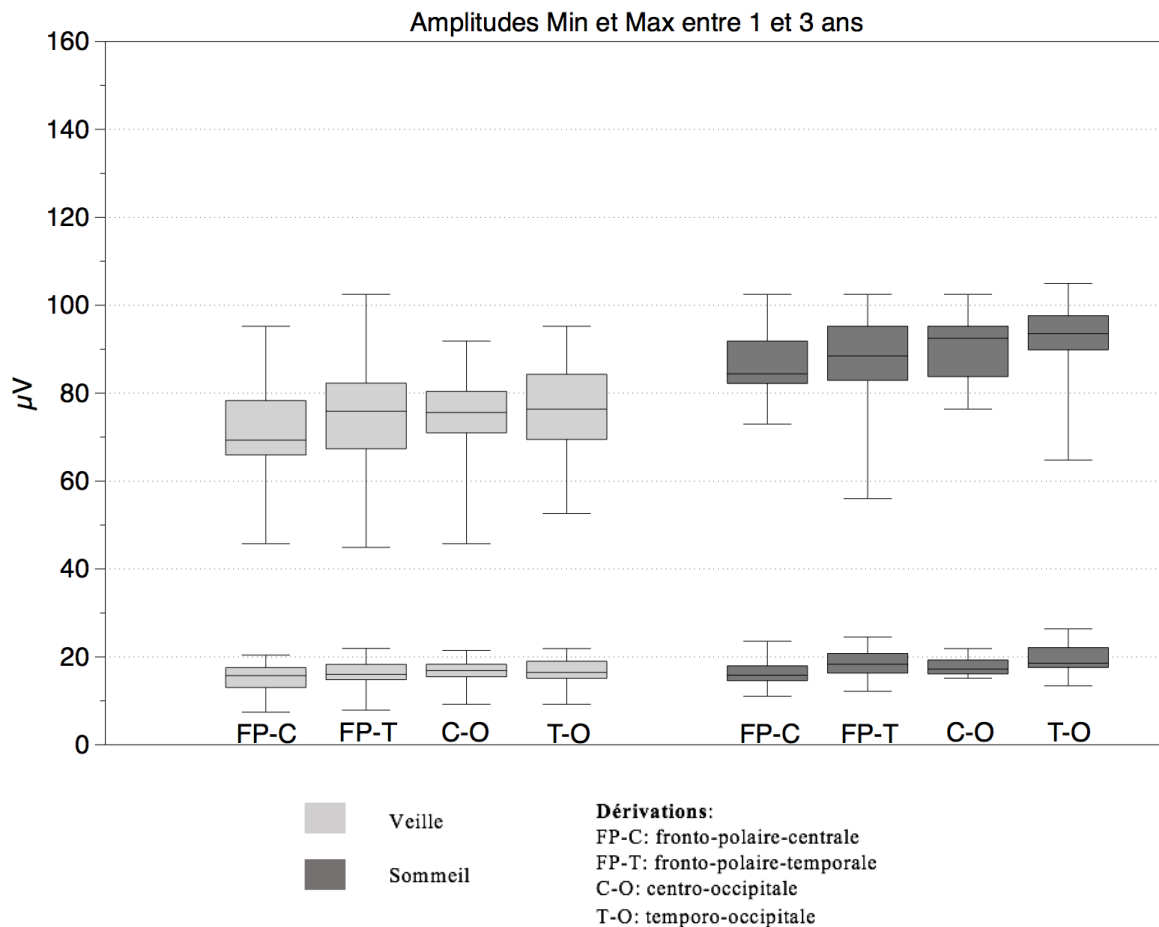


TABLEAU V : Normes aEEG 3 ans-6 ans

3 ANS-6 ANS	VEILLE		SOMMEIL	
	marge supérieure	marge inférieure	marge supérieure	marge inférieure
Fp1-C3	85,86 (79,08-93,36)	15,79 (15,09-16,42)	114,47(112,07-143,28)	21,31 (21,04-22,15)
Fp2-C4	86,25 (79,95-103,89)	16,67 (15,33-17,40)	120,01 (116,53-137,38)	21,60 (19,43-22,83)
Fp1-T3	81,16 (76,53-97,81)	15,50 (14,64-16,65)	104,82 (100,21-107,71)	22,64 (22,44-22,65)
Fp2-T4	84,88 (74,06-89,84)	16,94 (13,98-18,81)	92,34 (91,28-120,92)	20,27 (20,11-22,29)
C3-O1	82,91 (73,13-88,72)	18,46 (16,83-20,66)	126,90 (109,08-144,97)	24,51 (23,03-26,15)
C4-O2	89,06 (80,91-96,73)	18,87 (16,76-20,40)	131,39 (109,42-149,99)	26,78 (25,01-28,93)
T3-O1	77,47 (69,44-85,51)	19,37 (16,77-20,27)	100,54 (92,37-123,75)	21,65 (14,98-23,62)
T4-O2	83,57 (75,37-95,08)	17,77 (16,19-19,59)	111,72 (104,67-141,51)	23,39 (22,80-23,61)
C3-C4	64,39 (51,53-72,34)	13,09 (11,90-14,21)	87,67 (79,61-124,48)	20,52 (18,47-20,89)

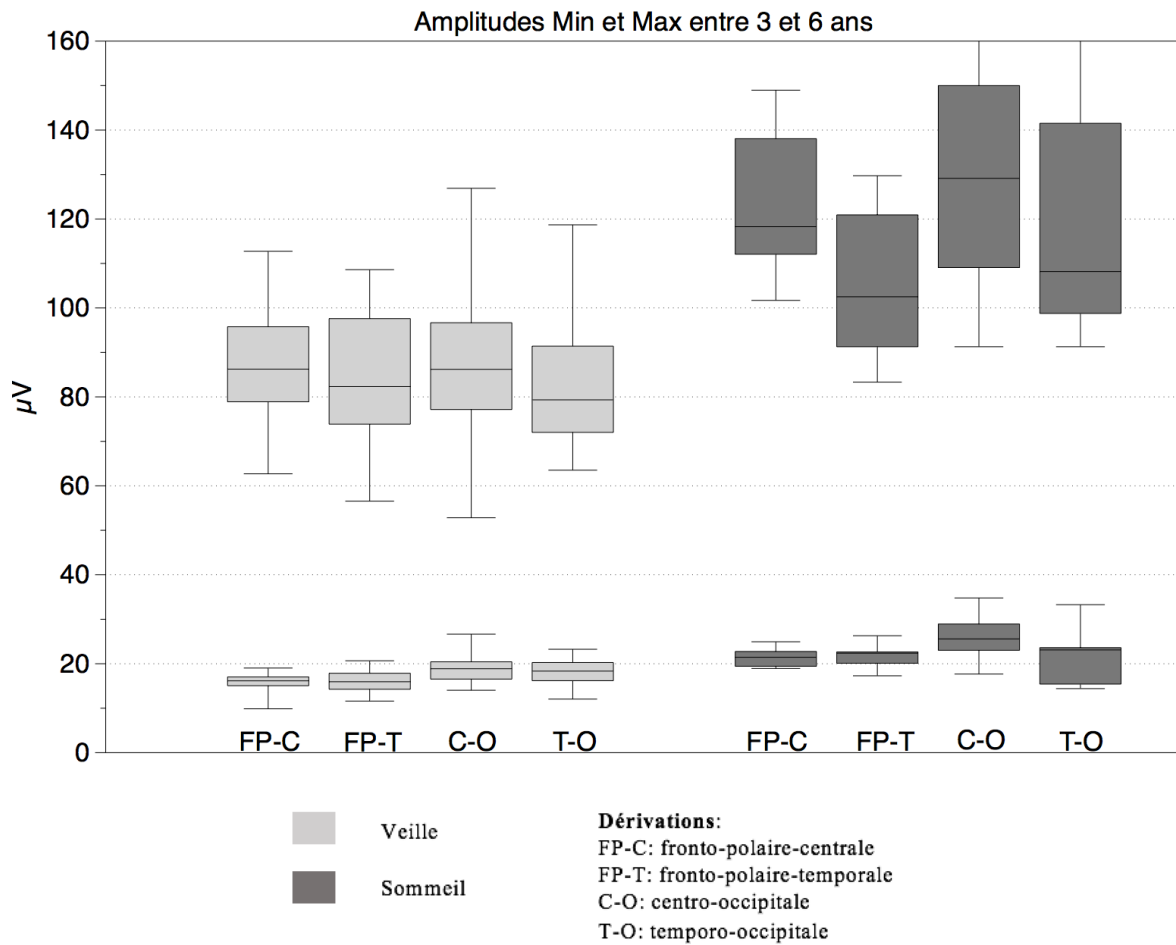


TABLEAU VI : Normes aEEG 6 ans-13 ans

6 ANS-13 ANS	VEILLE		SOMMEIL	
	marge supérieure	marge inférieure	marge supérieure	marge inférieure
Fp1-C3	80,03 (68,98-85,25)	16,07 (14,50-17,68)	87,36 (81,54-101,07)	15,56 (15,45-17,41)
Fp2-C4	69,44 (64,04-84,91)	16,67 (14,50-18,12)	93,79 (77,65-111,13)	14,77 (13,95-15,23)
Fp1-T3	69,44 (60,56-80,03)	14,78 (12,71-17,20)	66,62 (62,70-82,69)	13,81 (13,37-15,58)
Fp2-T4	68,98 (59,46-74,35)	15,62 (12,81-16,72)	61,73 (59,38-71,95)	13,22 (12,93-14,29)
C3-O1	85,73 (78,78-97,75)	20,15 (16,82-21,56)	92,06 (86,97-100,87)	18,30 (16,78-19,45)
C4-O2	88,74 (70,31-96,52)	18,82 (16,82-21,56)	92,63 (82,05-104,01)	18,24 (17,25-19,26)
T3-O1	79,92 (68,98-92,84)	18,12 (15,94-20,52)	76(66-84,51)	15,87 (14,43-16,81)
T4-O2	85,73 (70,32-97,42)	19,22 (15,84-20,61)	76,91 (69,30-85,16)	15,29 (14,06-16,63)
C3-C4	51,25 (46,91-67,39)	11,60 (10,07-13,46)	73,70 (62,68-85,91)	12,80 (12,28-14,34)

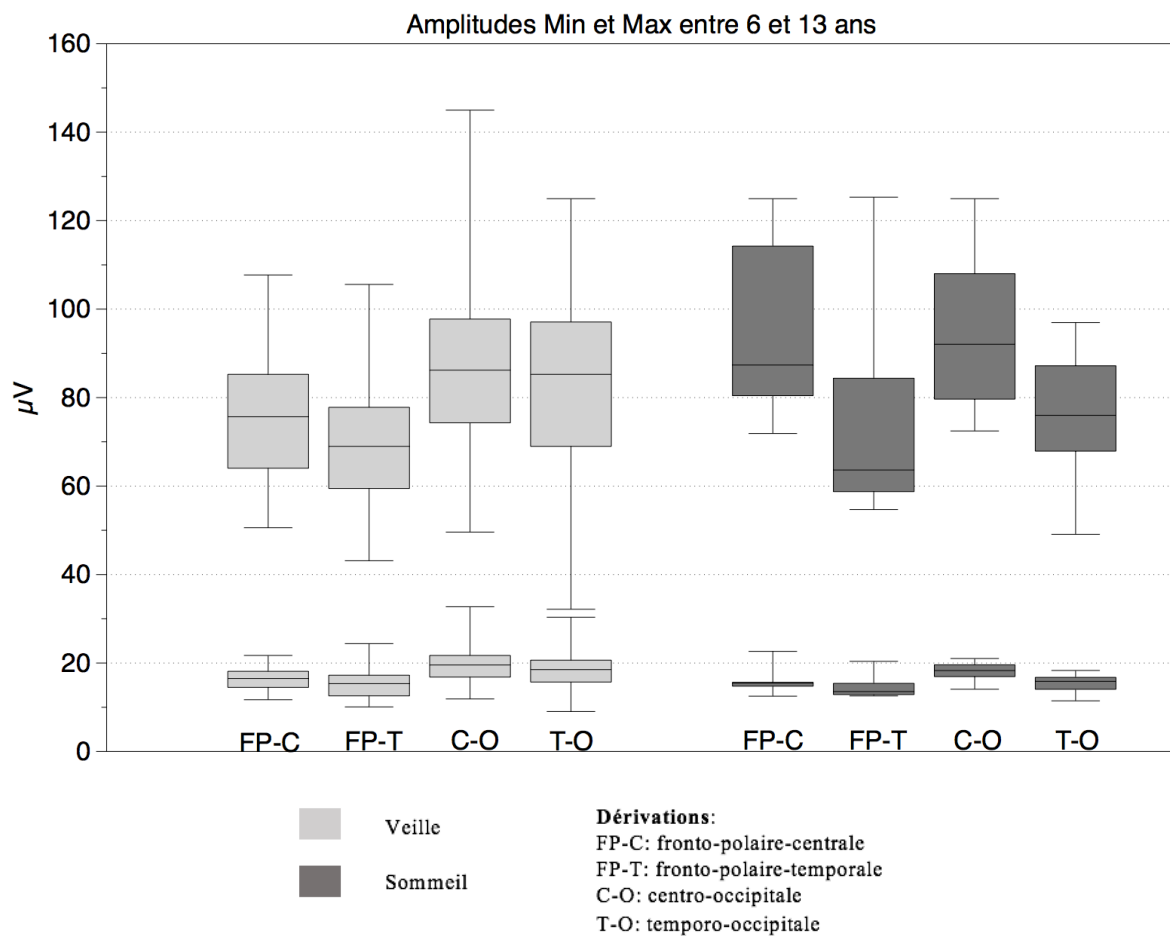
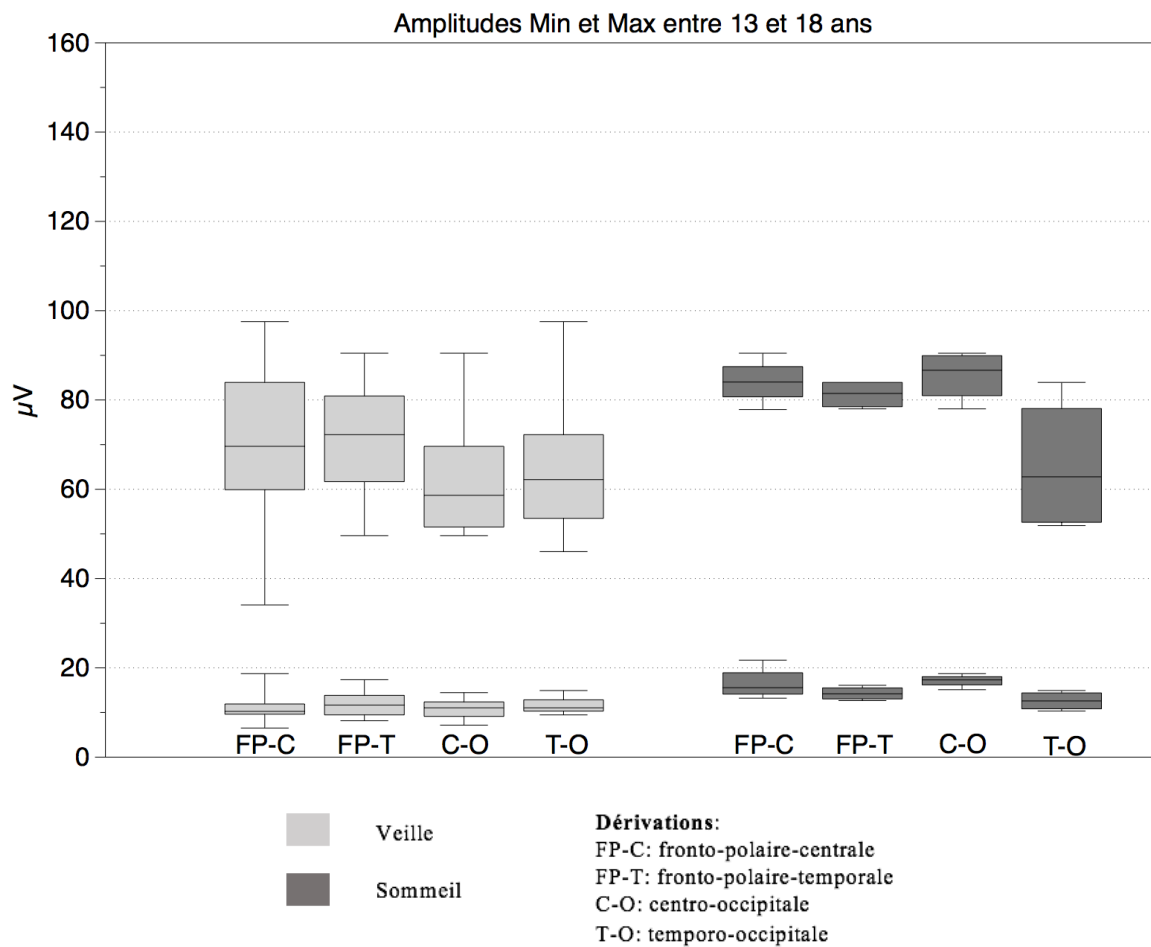
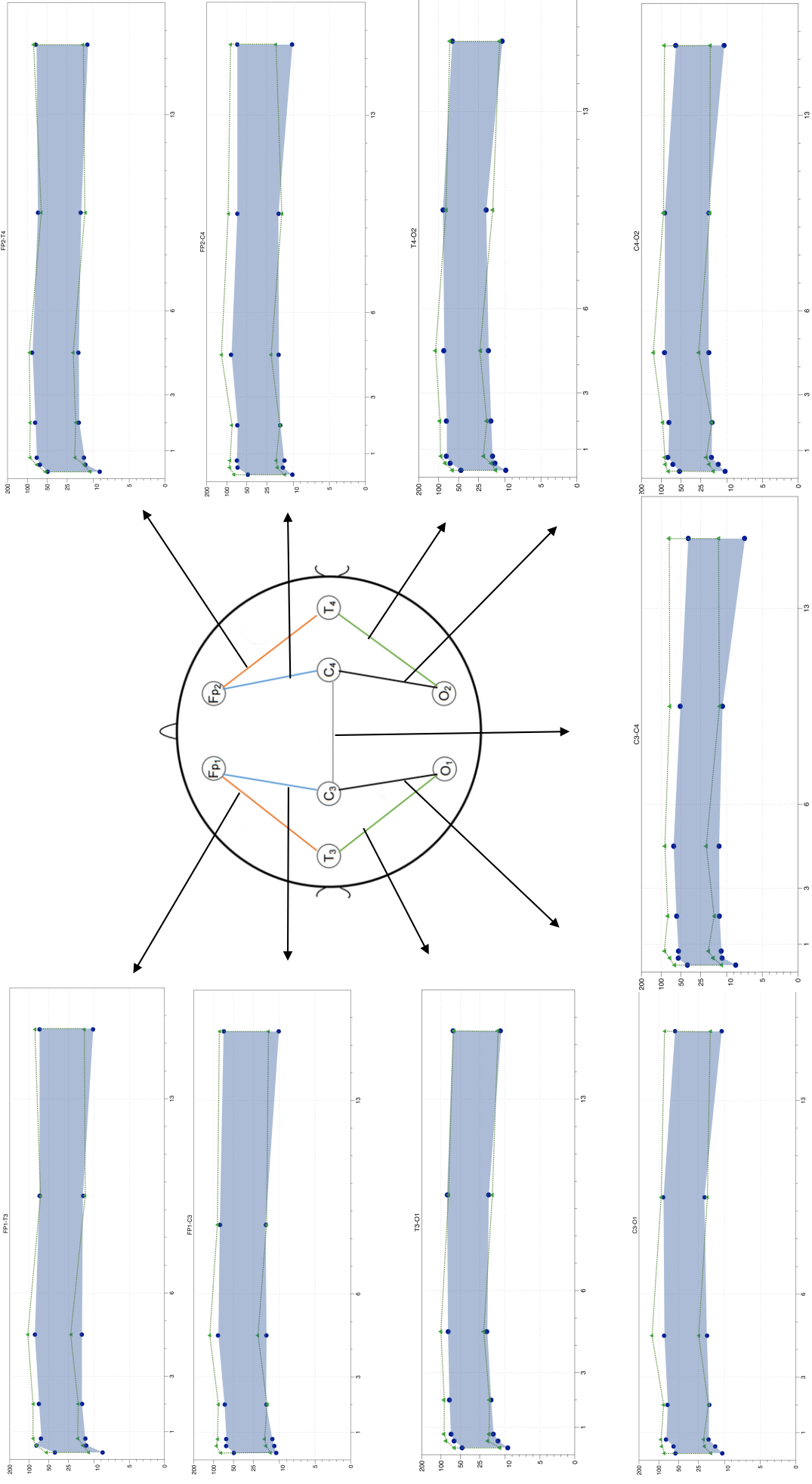


TABLEAU VII : Normes aEEG 13 ans-18 ans

13 ANS-18 ANS	VEILLE		SOMMEIL	
	marge supérieure	marge inférieure	marge supérieure	marge inférieure
Fp1-C3	70,02 (58,79-83,93)	10,25 (10,23-11,91)	80,71 (79,28-82,13)	14,65 (13,93-15,37)
Fp2-C4	69,62 (63,36-81,01)	10,45 (9,54-11,91)	87,48 (85,98-88,97)	18,37 (16,69-20,04)
Fp1-T3	69,62 (59,60-76,45)	10,42 (9,54-12,65)	81,46 (80,22-82,69)	14,17 (13,80-14,55)
Fp2-T4	75,05 (68,31-82,41)	12,38 (9,95-14,65)	80,97 (79,49-82,45)	14,37 (13,52-15,23)
C3-O1	56,56 (53,49-64,68)	11,05 (9,30-12,14)	80,97 (79,49-82,45)	16,23 (15,67-16,78)
C4-O2	60,23 (49,62-69,72)	11,05 (9,37-12,40)	89,93 (89,65-90,20)	17,98 (17,62-18,34)
T3-O1	64,95 (53,49-73,64)	11,87 (10,13-12,84)	62,79 (58,07-67,51)	13,13 (12,23-14,02)
T4-O2	62,16 (55,52-68,31)	11,05 (10,88-11,91)	67,89 (59,87-75,91)	12,09 (11,21-12,97)
C3-C4	38,60 (31,62-39,61)	7,50 (6,20-8,48)	76 (72,04-79,97)	13,23 (12,39-14,08)





Représentation graphique des normes aEEG de 1 mois à 18 ans, pour chaque dérivation

Article

Amplitude-Integrated EEG Monitoring in Pediatric Intensive Care: Prognostic Value in Meningitis before One Year of Age

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Abstract: Pediatric morbidity from meningitis remains considerable. Preventing complications is a major challenge to improve neurological outcome. Seizures may reveal the meningitis itself or some complications of this disease. Amplitude-integrated electroencephalography (aEEG) is gaining interest for the management of patients with acute neurological distress, beyond the neonatal age. This study aimed at evaluating the predictive value of aEEG monitoring during the acute phase in meningitis among a population of infants hospitalized in the pediatric intensive care unit (PICU), and at assessing the practicability of the technique. AEEG records of 25 infants younger than one year of age hospitalized for meningitis were retrospectively analyzed and correlated to clinical data and outcome. Recording was initiated, on average, within the first six hours for $n = 18$ (72%) patients, and overall quality was considered as good. Occurrence of seizure, of status epilepticus, and the background pattern were significantly associated with unfavorable neurological outcomes. AEEG may help in the management and prognostic assessment of pediatric meningitis. It is an easily achievable, reliable technique, and allows detection of subclinical seizures with minimal training. However, it is important to consider the limitations of aEEG, and combine it with conventional EEG for the best accuracy.

Keywords: meningitis; cerebral monitoring; seizure; amplitude-integrated electroencephalography; neurological outcome; pediatric critical care; infant



Citation: Beck, J.; Grosjean, C.; Bednarek, N.; Loron, G. Amplitude-Integrated EEG Monitoring in Pediatric Intensive Care: Prognostic Value in Meningitis before One Year of Age. *Children* **2022**, *9*, 668. <https://doi.org/10.3390/children9050668>

Academic Editor: Michael M. Hermon

Received: 5 April 2022

Accepted: 1 May 2022

Published: 5 May 2022

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1. Introduction

Acute meningitis is a major health problem around the world. Regardless of age, its global prevalence exceeded 10 million cases each year [1]. In 2017, considering infants under one year of age, incidence was estimated around 0.11% [2]. In newborns, incidence ranges from 0.22 to 0.5 per 1000 live births, even higher in premature and low birth weight infants, being at 20.4% and 13.6%, respectively [3–6].

In France, from 2010 to 2014, the predominant bacterial species were *Streptococcus agalactiae* (57.2%) and *Escherichia coli* (16.8%) in infants less than two months of age. *Streptococcus pneumoniae* (44%) and *Neisseria meningitidis* (31.7%) were predominant in infants between 2 and 12 months of age.

The mortality rate for bacterial meningitis in children is around 6.6% overall, and 7.2% under one year [7]. Neonatal meningitis mortality accounted for 6% to 8% of overall causes of death in children under five years of age in 2015 [8].

Because of the high risk of morbidity and death caused by meningitis, its diagnosis and treatment are an emergency. The risk factors for mortality are multiple: the severity of the infection, delay in diagnosis, prematurity, low weight for gestational age, seizures, and mechanical ventilation [4,5,9,10]. Furthermore, due to the disease and its complications, up to one third of survivors will develop permanent sequelae, such as epilepsy; encephalopathy;

motor or cognitive disorders; neurosensory sequelae (deafness, visual disorders); learning disabilities; and behavioral, language, or coordination disorders [4,11,12]. Brain imaging is performed to detect complications such as cerebral abscesses, ventriculitis, hydrocephaly, or strokes [4,10]. Imaging abnormalities are one of the risk factors for developing neurological sequelae [12].

The symptoms suggestive of meningitis vary according to age and the germ involved. Typically, the younger the child is, the less specific the signs will be. Digestive symptoms, irritability, or lethargy should suggest the diagnosis, but fever or a bulging fontanel may be absent in half or two thirds of cases, respectively [13,14]. Any delay in the start of suitable antibiotics will result in poorer outcome [15,16]. In most cases, meningitis is revealed by markers of severity or even complications such as seizures in approximately 20–30% of patients [6,9,13,17]. Reported risk factors for seizures are meningitis due to *Streptococcus pneumoniae*, imaging abnormalities, and immunosuppression [18]. Seizures are associated with in-hospital mortality and poor outcome [18]. However, seizures may be predominantly subtle or subclinical, especially among the youngest, which is why paraclinical examinations, such as brain monitoring, are helpful for early recognition.

The electroencephalogram (EEG) is the gold standard for detecting and characterizing seizures or epilepsy, and should be used in all cases of observed or suspected seizures [19,20]. However, continuous EEG monitoring is not available at any time in every neonatal or pediatric intensive care unit. Indeed, it requires trained and specially-skilled caregivers to accurately set the complex equipment, and then to interpret the full wealth of the trace. In order to cover all of the needs, the examinations are often limited from 30 to 60 min [20,21]. To enhance these accurate, but frequently intermittent, records, clinicians need trends that they can analyze continually.

The amplitude EEG (aEEG) was developed in the late 1960s by Douglas Maynard at the London Hospital, United Kingdom, for continuous monitoring of brain activity in adult, resuscitated patients [22]. Through amplitude-integrated EEG processing, the raw-EEG signal from one or two derivations is filtered, rectified, sampled, and then displayed at a slow speed (around 3.5 h per screen width). AEEG emphasizes the amplitude change of low frequency wavelengths over time. With minimal training, aEEG can be set and interpreted by a caregiver at bedside [23]. The recognition of background pattern and electrical seizures is quite good, as well as inter-observer reliability [21,23,24]. An extensive literature has pointed out the many advantages of aEEG in monitoring neonatal encephalopathy, neonatal seizures, and preterm birth. It has shown some value in older children, admitted for convulsive disorder, circulatory arrest, or cardiac surgery [19,25–35].

The relevance of aEEG in infectious meningitis in infants has not been reported yet.

This study aimed at assessing the predictive value of aEEG monitoring in infectious meningitis among children under one year of age. We hypothesized that it may help for early prediction of the neurologic outcome.

2. Materials and Methods

2.1. Study and Population

The aEEG was introduced in our pediatric and neonatal intensive care units in 2004, complementary to the use of standard EEG. We primary use aEEG when the EEG is not available (i.e., at nights and weekends), and for brain monitoring over days.

The present study was monocentric, observational, and took place over a period of 10 years. We retrospectively included patients with the following criteria: infants less than one year old; hospitalized between 1 January 2004 and 31 December 2014, in neonatal or pediatric intensive care units at the tertiary University Hospital of Reims with infectious meningitis; and available aEEG recording. During this period, the neonatal and pediatric intensive care units were combined into one polyvalent unit, which made the collection of records easier, and after 2017, due to the introduction of mandatory vaccination of children in France, the number of cases included could have been smaller.

2.2. Demographic, Clinical, and Biological Data

The Medical Information Department screened patients meeting the inclusion criteria. Demographical data extracted from medical charts included: sex, age (in days), weight (kg), height (cm), head circumference (cm), and history of neurological pathology (epilepsy, febrile seizure, encephalopathy, other).

Data characterizing meningitis were: neurological signs (tone, consciousness, bulging fontanel, seizures), fever, altered general condition, vomiting or feeding impairment, respiratory or hemodynamic distress, time from onset of symptoms to diagnosis of meningitis, inflammatory markers (CRP (mg/L), PCT (ng/mL)), initial leukocytes (/mm³), bacteriological tests (blood culture, cerebrospinal fluid test with cell count, protein, glucose, lactate, and culture), and antimicrobial therapy (type, start-up time, and duration).

Data concerning outcome were death, diagnosis of epilepsy, subsequent encephalopathy (yes/no), radiological brain injuries, and duration of hospital and PICU stay. The Pediatric Cerebral Performance Category (PCPC) was assessed one year after hospital discharge, according to Utstein's guidelines [36–39]. This scale is used to categorize the child's deficit and disability in six levels: 1/normal, 2/mild disability, 3/moderate disability, 4/severe disability, 5/vegetative state or coma, 6/death. For statistical purposes, PCPC scores were categorized as "favorable outcome group" (scores 1–2–3) versus "unfavorable outcome group" (PCPC score 4–5–6). The PCPC score presented in this study is available in Supplementary Materials (Table S1).

2.3. aEEG Data

The aEEG traces were recorded with one of these devices: Olympic CFM[®]6000 (three electrodes), Olympic Brainz Monitor[®], or Brainz BMR3 Monitor[®], and read on the device or analyzed using the "Analyze Research" software for Brainz Monitors[®] (all those devices and software are distributed by Natus[®], Middleton, WI, USA).

aEEG traces were characterized as follow: duration (min), quality of the recordings, lower and upper margin, presence of modulation, presence and number of electrical seizures, time from beginning to first continuous trace, and time from beginning to first modulation. Background pattern was classified according to the classification popularized by Hellström-Westas as: continuous normal, discontinuous, burst-suppression, continuous low-voltage, flat trace [40].

The background was scored from 1 to 4 points, for every 3-h period, for the first 24 h of recording, as previously reported [41]. Normal trace, discontinuous trace, burst-suppression, and low-voltage trace and flat trace accounted for 1, 2, 3, and 4 points, respectively. The score could therefore range from 8 to 32, with higher scores reflecting a more impaired background activity. If the 24 h period was not complete, the score of the last recorded period was repeated for the following missing periods.

Electrical seizures on aEEG were acknowledged by notches on the amplitude trace and evocative, rhythmic pattern on the continuous EEG.

Figure S1 illustrates an example of seizure on the aEEG trace used in this study, and Figure S2 is an example of artifacts caused by ventilation. They are available in Supplementary Materials.

Recording quality was evaluated according to the impedance value and its variations.

Each trace was interpreted by a pediatric resident recently trained in aEEG interpretation (JB), and blindly reviewed by a hospital clinician experienced in aEEG interpretation (GL).

2.4. Statistics

Statistical analyses were performed using STATA 16 software (Stata/SE version, STATA-Corp LLC, 4905 Lakeway Drive, College Station, TX 77845-4512, USA). Descriptive statistics were expressed using standard deviation \pm means for quantitative variables, and percentage (%) for qualitative variables. Univariate analyses were performed with Student's T test for quantitative variables, and chi-squared test or Fisher's exact test for qualitative variables. The strength of statistical association was performed by measuring

the odds ratio and its 95% confidence interval (CI) for the predictivity of neurological prognosis by aEEG items. The predictive value of the background trace obtained by aEEG during the first 24 h of recording was plotted on an ROC curve with the 95% CI of it. Threshold for significance was defined as $p < 0.05$.

3. Ethics Conflict of Interest

There are no conflicts of interest to report. The present study was declared to an independent French administrative authority named “Commission Nationale de l’Informatique et des Libertés” (CNIL), in 5 October 2016 under the declaration number 1995487 v0.

4. Results

4.1. Population and Epidemiological Characteristics

We identified 35 patients less than 1 year old, admitted to the pediatric and neonatal intensive care units with acute meningitis, whose medical charts reported an aEEG monitoring. For 10 children, the original aEEG trace was unusable because of technical issues or missing data. The demographic characteristics of these 10 patients did not differ from the subsequently included patients (data not shown) (Figure 1). Of the 25 children finally included in the study, 18 of them (72%) were classified in the favorable outcome group (PCPC 1–2–3), and 7 (28%) in the unfavorable outcome group (PCPC 4–5–6) (Table 1).

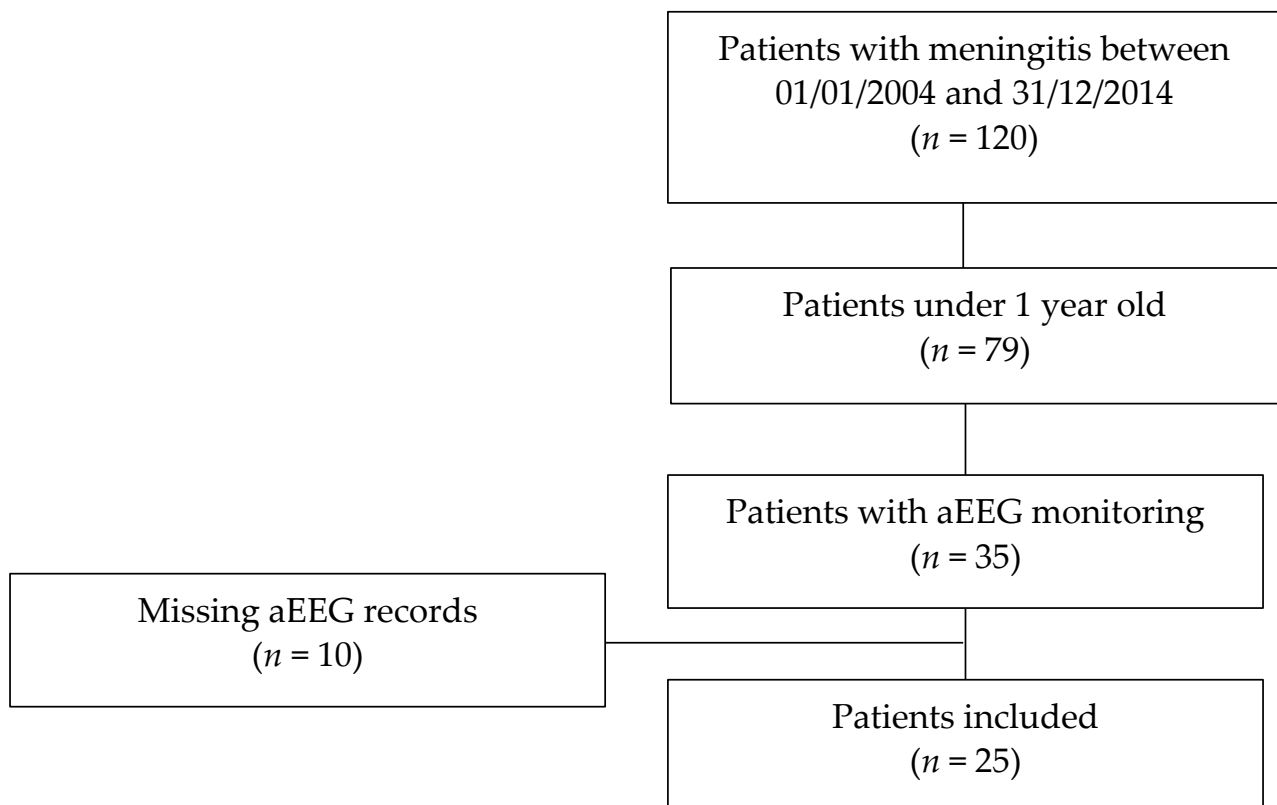


Figure 1. Flow chart of the study. Thirty-five patients met the inclusion criteria: hospitalization in pediatric or neonatal intensive care unit; age: less than one year; aEEG monitoring reported in medical charts. Ten patients were subsequently excluded due to missing the complete aEEG dataset.

Table 1. Characteristics of patients included in the study, according to outcome scores.

Patient Characteristics	Favorable Outcome Group <i>n</i> = 18	Unfavorable Outcome Group <i>n</i> = 7	<i>p</i>
Sex ¹ , men	10 (55.6)	2 (28.6)	0.378
Age ³ , days	22 ± 60	61 ± 86	0.471
Weight ² , g	3732 ± 1494	4187 ± 2837	0.602
Size ² , cm	51.6 ± 7.0	52.9 ± 9.4	0.725
Head circumference ² , cm	35.6 ± 4.5	36.2 ± 5.5	0.795
Center referring the patient to intensive care			
○ University hospital ¹	6 (33.3)	1 (14.3)	0.626
○ Other hospital ¹	12 (66.7)	6 (85.7)	
Neurological history ¹	2 (11.1)	0 (0)	1

¹ Data expressed in *n* (%). ² Continuous variables expressed as averages ± standard deviation. ³ Variables expressed in median ± interquartile range.

Two patients presented with a previous neurological history: one had hydrocephalus, and the second had spina bifida.

4.2. Characteristics of Meningitis and Hospitalization

The mean time from symptom onset to diagnosis was 38.65 ± 33.48 h for 80% of the patients (*n* = 20). For the remaining 20% (*n* = 5), the time was not clearly indicated, but was less than 12 h for each of them. The mean time between the onset of symptoms and the start of anti-microbial therapy was 23.2 ± 15.75 h for 80% of patients (*n* = 20). For 16% (*n* = 4) of the patients, the time was not clearly indicated, but was less than 12 h for each patient, and the time was not specified for 4% of the patients (*n* = 1).

Patients in the unfavorable outcome group had a significantly higher initial PCT than the favorable outcome group. The unfavorable outcome group had a significantly higher cerebrospinal protein concentration on the first lumbar puncture.

Mean time for parenteral nutrition weaning and the length of stay in PICU were significantly increased in the unfavorable outcome group. The meningitis and hospitalization characteristics of the cohort are described in Table 2.

4.3. Patient Outcome

Two (8%) patients died. Twelve patients (52%) developed clinical neurological and sensorial sequelae. Three patients (13%) were diagnosed with epilepsy, and one (4%) with encephalopathy at discharge. Brain injuries were reported for 16 patients (64%), divided into ventricular enlargement (*n* = 5 (20%)), multifocal injury of the parenchyma (*n* = 3 (12%)), extracerebellar collection (*n* = 3 (12%)), and brain injuries involving several of these locations (*n* = 5 (20%)).

4.4. Description and Prognostic Value of aEEG

Median time to start aEEG recording was 3 ± 5.5 h after the beginning of the PICU hospitalization, for a mean duration of 99.34 ± 83.21 h, with a significant difference between the two groups, with longer recordings for the unfavorable outcome group with 161.43 ± 102.29 versus 75.19 ± 62.27 h (*p* = 0.016). Distribution of the aEEG recordings over time is presented in Figure 2. However, there was no significant difference between the two groups regarding the quality of the record (unfavorable group 83.34 ± 22.60 versus favorable group 84.01 ± 21.91%, *p* = 0.946). Furthermore, we noted that aEEG recordings were of sufficient quality to be interpreted for 84% of the recordings.

Table 2. Bacteriological, clinical, and management characteristics of recorded patients, according to outcome.

Characteristics ¹	Favorable Outcome Group <i>n</i> = 18	Unfavorable Outcome Group <i>n</i> = 7	<i>p</i>
Symptoms ¹			
Neurologicals exclusive *	1 (5.6)	1 (14.3)	0.518
Generals exclusive **	6 (33.3)	1 (14.3)	
Both	11 (61.1)	5 (71.4)	
Time from symptoms onset to diagnosis ²	37.1 ± 35.8	45 ± 24.7	0.683
<12 h ¹	5 (27.8)	3 (42.9)	0.853
12–24 h ¹	5 (27.8)	2 (28.6)	
>24 h ¹	8 (44.4)	2 (28.6)	
Initial CRP (mg/mL) ²	78.1 ± 101.5	150.9 ± 118.9	0.138
Initial PCT (ng/mL) ² (0 ³ /4 ³)	25.8 ± 22.8	117.3 ± 48.1	<0.001
Initial leukocytes (/mm ³) ²	9.0 ± 7.6	6.4 ± 5.1	0.414
Positive blood culture ¹	12 (66.7)	5 (71.4)	0.819
First lumbar puncture ¹	17 ***	7	-
Leukocytes (/mm ³) ² (3 ³ /0 ³)	2055.9 ± 2341.1	4135.9 ± 7357.4	0.323
Hematite (/mm ³) ² (4 ³ /1 ³)	2829.1 ± 4978.5	4963.7 ± 3049.4	0.459
Proteins (g/l) ² (3 ³ /0 ³)	2.41 ± 1.57	5.08 ± 2.60	0.007
Glucose (g/l) ² (3 ³ /0 ³)	1.39 ± 1.13	0.62 ± 0.96	0.138
Lactates (mmol/l) ² (8 ³ /4 ³)	6.54 ± 4.28	10.53 ± 2.22	0.156
Chlore (mmol/l) ² (4 ³ /3 ³)	115.5 ± 5.8	109.3 ± 8.4	0.104
Cerebrospinal fluid sterile ¹	2	0	
Positive bacteriology ¹	15	7	
○ Streptococcus B			
○ Pneumococcus	4 (22.2)	2 (28.6)	
○ Enterovirus	5 (27.8)	2 (28.6)	
○ Enterococcus faecalis	1 (5.5)	0	
○ Escherichia Coli K1	1 (5.5)	0	
○ Meningococcus B	2 (11.1)	3 (42.9)	
○ Coagulase negative staphylococci	1 (5.5)	0	
Time from initial symptoms to treatment ¹			
<6 h	5 (27.8)	2 (28.6)	1
6–12 h	4 (22.2)	2 (28.6)	
>12 h	8 (44.4)	3 (42.9)	
Total duration of antibiotic ^{2,4}	16.7 ± 8.1	20.3 ± 6.6	0.305
Mean time of parenteral nutrition weaning ^{2,4} (1 ³ /3 ³)	4.3 ± 7.1	13.6 ± 12.5	0.043
Length of stay in PICU ^{2,4}	7.9 ± 4.7	20.4 ± 5.9	<0.001
Total length of stay at hospital ^{2,4}	22.4 ± 22.1	33.0 ± 12.7	0.247

¹ Data in *n* (%). ² Continuous variables expressed as averages ± standard deviation. ³ N missing data. ⁴ Data expressed in days. * The patient in the favorable outcome group had a bulging fontanel; this one in the unfavorable outcome group had seizures. ** Patients had fever, vomiting or feeding impairment, purpura, respiratory or hemodynamic distress. *** One patient did not undergo a lumbar puncture because of an exudative dysraphism, but had a positive blood culture for streptococcus B.

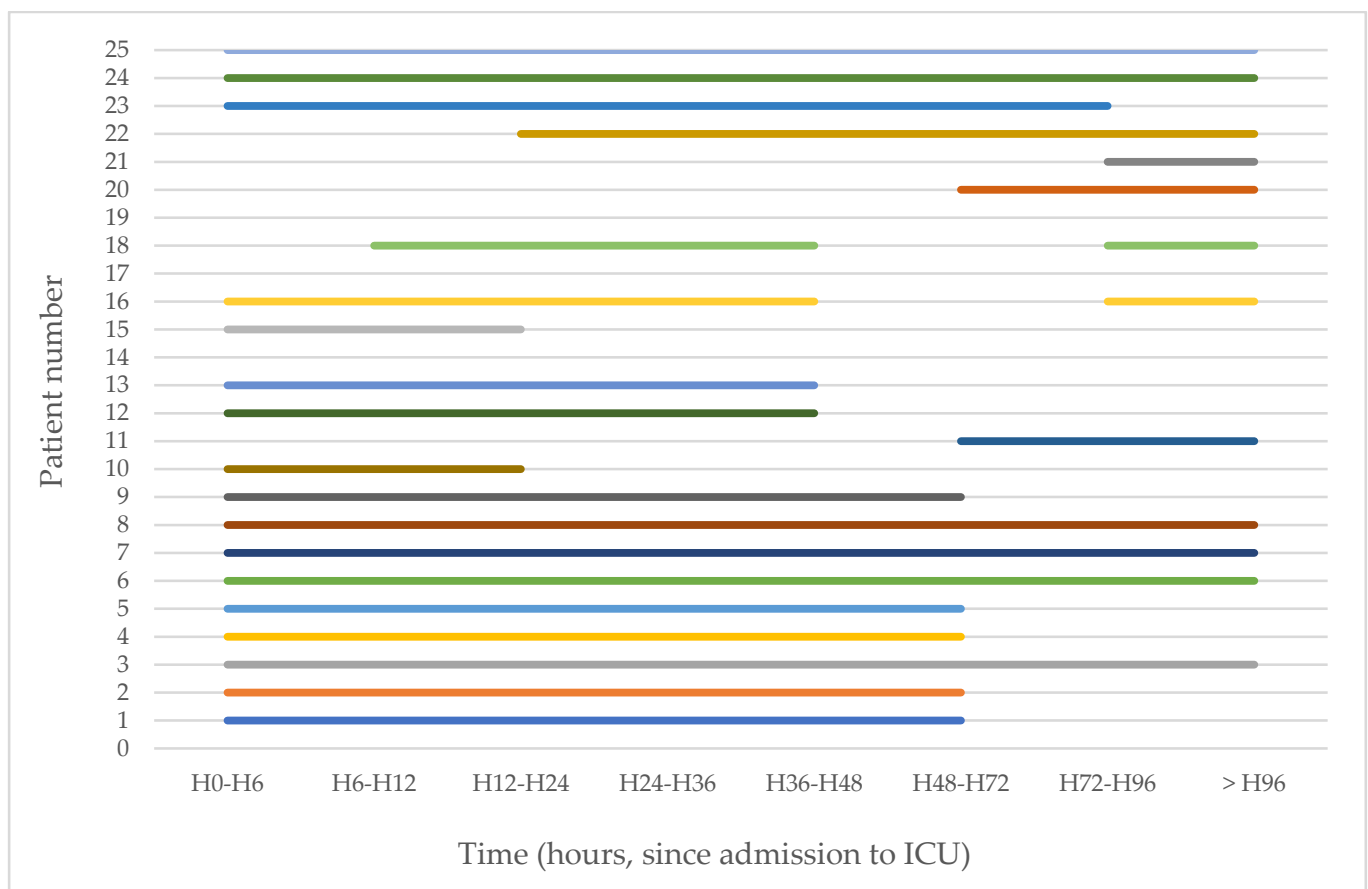


Figure 2. Distribution over time of aEEG recordings for each patient (1–25). Patients 14, 17, and 19: aEEG was recorded beyond H96 of hospitalization in ICU.

Looking for seizures, 202 notches were reported and analyzed on aEEG traces for the 25 patients. Forty-eight (24%) notches were considered as artifacts. Diagnosis of electrical seizure was validated on aEEG for 154 (76%) notches, accounting for 11 patients overall (44%). Among them, two infants (18%) presented electrical seizures only (four seizures each). Nine patients (82%) presented both clinical and infraclinical seizures during recordings. They presented a median number of 5 ± 12 electrical seizures only (from 2 to 69 seizures). Overall, 18 infants (72%) presented clinical or electrical seizures at any moment of their disease.

Seizures and electrical status epilepticus were more frequent in the unfavorable outcome group compared to the favorable outcome group. The presence of electrical seizures and electrical status epilepticus was significantly observed within the unfavorable outcome group.

The first 24-h background pattern score was significantly higher in the unfavorable outcome group compared to the favorable outcome group.

We did not document a significant difference for the “recovery of a normal background” or the “recovery of a modulation”, whose details are presented in Table 3. The predictive value of the background score at aEEG is presented by the ROC curve in Figure 3, with an area under the curve of 0.758 (95% CI 0.521–0.995). The cut-off point for the best sensitivity–specificity “couple” is a background tracing score of 11; when it is ≥ 11 , the sensitivity was 71.43% and the specificity was 66.67% for an unfavorable prognosis by the PCPC scale (4–6).

Table 3. Prognostic value of aEEG features during recording.

aEEG Characteristics	Favorable Outcome Group (n = 18)	Unfavorable Outcome Group (n = 7)	p	OR (IC 95%)
Background score over first 24 h ²	10 ± 3	16 ± 7	0.008	
<161	15 (83.3)	3 (42.9)	0.066	6.66 (0.95–46.56)
≥161	3 (16.7)	4 (57.1)		
Electrical epileptic seizures ¹				
Yes	5 (27.8)	6 (85.7)	0.021	15.60 (1.48–164.38)
No	13 (72.2)	1 (14.3)		
Electrical status epilepticus ¹				
Yes	2 (11.1)	5 (71.4)	0.007	20 (2.21–180.90)
No	16 (88.9)	2 (28.6)		
Recovery of a modulation ¹				
Yes	15 (83.3)	5 (71.4)	0.597	0.5 (0.06–3.91)
No	3 (16.7)	2 (28.6)		
Recovery of a normal background ¹				
Yes	11 (61.1)	1 (14.3)	0.073	0.11 (0.01–1.08)
No	7 (38.9)	6 (85.7)		

¹ Data in n (%). ² Continuous variables expressed as averages ± standard deviation.

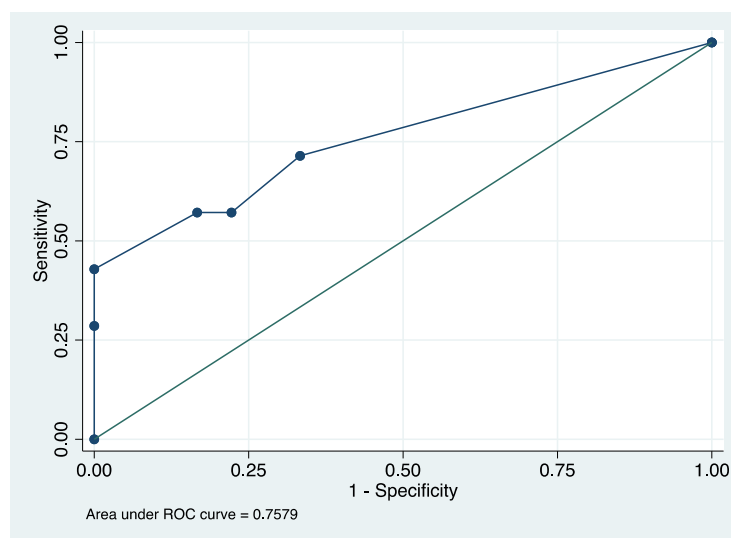


Figure 3. Predictive value of the background score at aEEG, ROC curve.

5. Discussion

To the best of our knowledge, this is the first report on the contribution of aEEG in meningitis in infants under one year of age. Brain monitoring by aEEG in meningitis is easy and helpful to prognosis.

We noticed the ability of aEEG monitoring in identifying subclinical seizures in this setting. Moreover, the presence of electrical seizures, status epilepticus, and a higher 24-h background pattern score were associated with an unfavorable outcome.

The impact of seizures during acute meningitis on neurologic outcome has already been highlighted. If seizures are prolonged, difficult to treat, or develop 72 h after admission, neurologic sequelae are more likely to occur, and could be suggestive of a cerebrovascular event [16]. Ouchenir et al. showed that the presence of seizures is a prognostic marker of

motor sequelae, developmental delay, and death [10]. Early detection and treatment of seizures is associated with a higher likelihood of seizure cessation [42]. AEEG could allow earlier detection of seizures, particularly subclinical ones, for a more reactive initiation of therapy, and to prevent them from persisting. Moreover, a recent study showed that the time elapsed from ICU admission to initiation of continuous EEG was associated with increased mortality [43]. A dedicated study would be needed for aEEG in patients with meningitis.

Here, electroencephalographic monitoring using aEEG documented a high prevalence of seizures in meningitis among infants, especially with few to no clinical signs in meningitis. Eighty-eight percent of seizures recognized at aEEG had no clinical substrate in the medical charts in our study. The retrospective design of this work and the absence of contemporary video recording did not help in identifying all clinical manifestations. However, the high prevalence of subclinical seizures in pediatrics in acute care has already been highlighted [44]. AEEG is even more accurate in the detection of infra-clinical status epilepticus than infra-clinical isolated seizures [26].

Compression of the trace, due to the amplitude-integrated processing, may limit the ability to detect seizures. In our experience, assessing the raw EEG when a notch is visualized on the aEEG background pattern is a crucial step which increases the probability of detecting single short seizures [45]. AEEG dedicated devices can display aEEG and raw EEG simultaneously. Bruns et al. reported a better seizure detection on aEEG rather than clinical observation, especially for non-convulsive status epilepticus [31]. Shellhaas et al. showed that aEEG did not increase the number of neuroimaging examinations nor the use of antiepileptic drugs, but that it allowed less overtreatment of children based only on clinical suspicions of seizures without electroencephalographic confirmation [29].

The clinical course and prognosis of meningitis observed in our study is in agreement with other studies on the subject. The mortality rate found in our study is comparable to that described in the literature among infants [7]. However, we reported fewer sequelae in the present work (16%) than the literature reported [4,11,12]. This can be explained by the limited population in our study. Yet, long term epilepsy occurred in 13% of our population, close to the observation of Ben Hamouda et al. and also Briand et al., during the follow-up at around 24 months [4,11].

Beyond meningitis, aEEG monitoring has proven prognostic accuracy in pediatrics and among adults. Thomas et al. showed that a moderately or severely altered background pattern, according to the classification of al Naqeeb [46], in the first 24 h of aEEG monitoring was associated with a prognosis of death for a positive predictive value of 77.8% [47]. In a more recent study (Bourgouin et al.), the background pattern during the first 24 hours after cardiac arrest was significantly associated with outcome [41]. This is corroborated in adults after cardiorespiratory arrest, with a poor prognosis in 66% of patients with a flat tracing in normothermia [48,49].

Poorly reactive background pattern on conventional EEG monitoring is associated with an adverse outcome [50,51]. In our study, we found a significantly higher 24-h background score in patients with an unfavorable neurological prognosis according to the PCPC, in agreement with other studies. A significant improvement of the background pattern was observed in the study of Gui et al. in infants who had been through or would go through a congenital heart disease surgery, as well as sleep–wake rhythm abnormalities associated with severe infections [52]. After cardiac surgery in infants less than three months of age, a lack of postoperative sleep–wake cycling predicted poorer motor and cognitive outcomes at one year of age [53]. Due to the heterogeneity in duration and timing of aEEG recordings, our study was probably not suitable to assess sleep–wake cycling prognosis value. This could be evaluated in the future.

One of the potential interests of aEEG is its ease of use. In the present work, we observed that aEEG was initiated within the first 6 h for 72% of patients. In pediatric and neonatal intensive care units, time to electroencephalography has been correlated to mortality [43]. This should be assessed using aEEG in a dedicated study. The aEEG monitor

is easy to set up and quickly available, even out of care hours, unlike the standard EEG in many centers [19]. In our day practice, we found that aEEG is a reliable and reproducible technique, and the aEEGs recordings were of sufficient quality for interpretation more than 80% of the time. Moreover, it can be used over long periods of time, as 56% of the patients were recorded for more than 72 h with a good quality of recording, corroborated by previous works [30,54]. However, aEEG has several limitations. In current dedicated devices, aEEG is recorded over one or two channels, whereas conventional EEG uses 10 to 20 channels by default. This results in poor spatial resolution, and less sensitivity to very localized phenomenon. Underestimation therefore seems inevitable, especially with single-lead monitors with an electrical seizure detection rate of about 40%. A two-lead monitor, as used in 80% of cases in our study, improves the sensitivity of detection of electrical seizures [55]. Kobayashi et al. associated the aEEG with the “spectral edge frequency” technique to increase the detection of seizures by staining the tracing [25].

AEEG monitoring requires training for setting and interpretation. A defective contact of the electrodes or a bad impedance makes the trace uninterpretable. In addition, misinterpretation of traces due to artifacts or lack of training can lead to an under- or overestimation of electrical seizures, and even an overuse of antiepileptic treatment [19,56]. According to Bruns’ study, non-expert recognition of seizures showed good sensitivity, but the high false-positive rate highlights the need for experts used to raw and conventional EEG [33]. Patient movements and care must be annotated on the trace because they induce artifacts that may lead to misinterpretation. In addition to seizures, left/right asymmetry of background can be interpreted relatively safely after a short training and indication of artifacts on the tracing [57,58]. However, the questions of how to interpret the background pattern in patients beyond the neonatal period and how to handle this interpretation arise. Indeed, there are various classifications applied to premature and newborns [46,59–61], but to date, there is no similar one for older patients [33].

The population of this study included patients from the neonatal period to one year of age, even if the diagnostic and therapeutic management was a little different between these two populations. We have therefore pooled them in this work due to the common susceptibility of their developing brain to produce seizures, and because they were hospitalized in the same intensive care unit.

Finally, our study had some limitations that may have somewhat impacted the validity of our results. It was a retrospective study with a small number of patients, and all of them required hospitalization in an intensive care unit, which may not be representative of the entire population of pediatric patients with meningitis. We can also assume that not all clinical seizures were recorded in the medical records, providing an information bias, which may have led to an overestimation of the number of subclinical seizures.

6. Conclusions

In conclusion, according to our results, and in agreement with the studies previously carried out on the use of the aEEG and the studies concerning meningitis, the aEEG is a promising and useful neurological monitoring tool during the evolution of severe meningitis in infants. The technique is reproducible, easy to set up, and reliable with minimal training in the interpretation of the tracings and the detection of electrical seizures. It should be used as soon as the diagnosis is established and from the beginning of care. This could improve management and allow a better assessment of the prognosis of severe meningitis in view of the statistically significant association found between the presence of seizures or convulsive state and a poor neurological prognosis.

However, the question remains whether a more reactive management of seizures would improve the prognosis of these patients. Brain monitoring with aEEG, offering a more comprehensive inventory of seizures, could be considered in a prospective work to provide some elements of the answer.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/children9050668/s1>, Table S1: The Pediatric Cerebral Performance Category Score; Figure S1: Example of seizure on amplitude-integrated EEG trace; Figure S2: Example of a ventilation artifact.

Author Contributions: Conceptualization, J.B., G.L. and N.B.; methodology, J.B. and G.L.; software, J.B.; validation, J.B., G.L., N.B. and C.G.; formal analysis, J.B.; investigation, J.B.; resources, J.B. and G.L.; data curation, J.B., G.L. and C.G.; writing—original draft preparation, J.B. and C.G.; writing—review and editing, J.B., G.L., N.B. and C.G.; visualization, J.B. and C.G.; supervision, G.L. and N.B.; project administration, J.B. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by an independent French administrative authority named “Commission Nationale de l’Informatique et des Libertés” (CNIL) in 2016, under the declaration number 1995487 v0.

Informed Consent Statement: Patient consent was waived due to the retrospective analysis of data collected as part of a routine care.

Data Availability Statement: Data available on request due to restrictions, e.g., privacy or ethical. The data presented in this study are available on request from the corresponding author.

Acknowledgments: We are grateful to the American Committee for its unwavering support of the American Memorial Hospital for over 100 years.

Conflicts of Interest: The authors declare no conflict of interest.

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FACULTE DE MEDECINE

ANNEE UNIVERSITAIRE 2017/2018

MEMOIRE DE DIPLOMES D'ETUDES SPECIALISEES

Spécialité : PEDIATRIE

Vélocimétrie par doppler Ultrafast® de la vascularisation cérébrale des grands prématurés en période néonatale.

Présenté par : MEUNIER Hélène

Soutenu le 4 Avril 2018 à Nancy

Directeur de mémoire : Dr Gauthier LORON

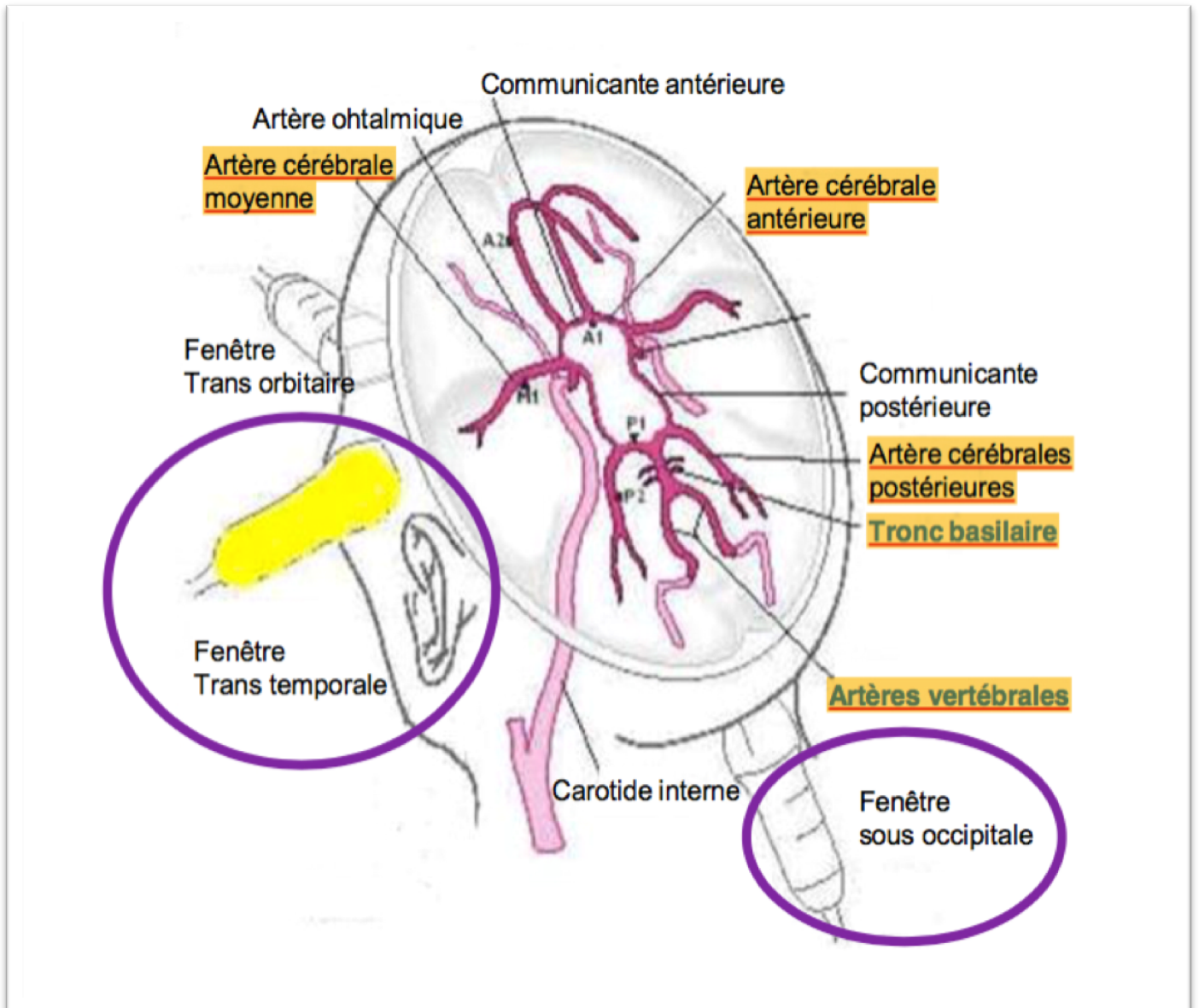


Figure 1.

Schéma représentant la position de la sonde d'échographie pour deux des trois fenêtres échographiques (temporale et sous occipitale). Surlignées en jaune les artères cérébrales étudiées et entourées en violet les deux fenêtres échographiques utilisées.



Figure 2.

Installation de l'échographe Aixplorer auprès de l'incubateur d'un prématuré (A). Positionnement de la sonde échographique selon la fenêtre échographique utilisée : fenêtre antérieure par la fontanelle bregmatique (B) pour l'artère cérébrale antérieure, fenêtre latérale gauche par voie transtemporale (C) pour les artères cérébrales moyenne et postérieure et fenêtre postérieure par voie sous occipitale (D) pour le tronc basilaire et les artères vertébrales droite et gauche.

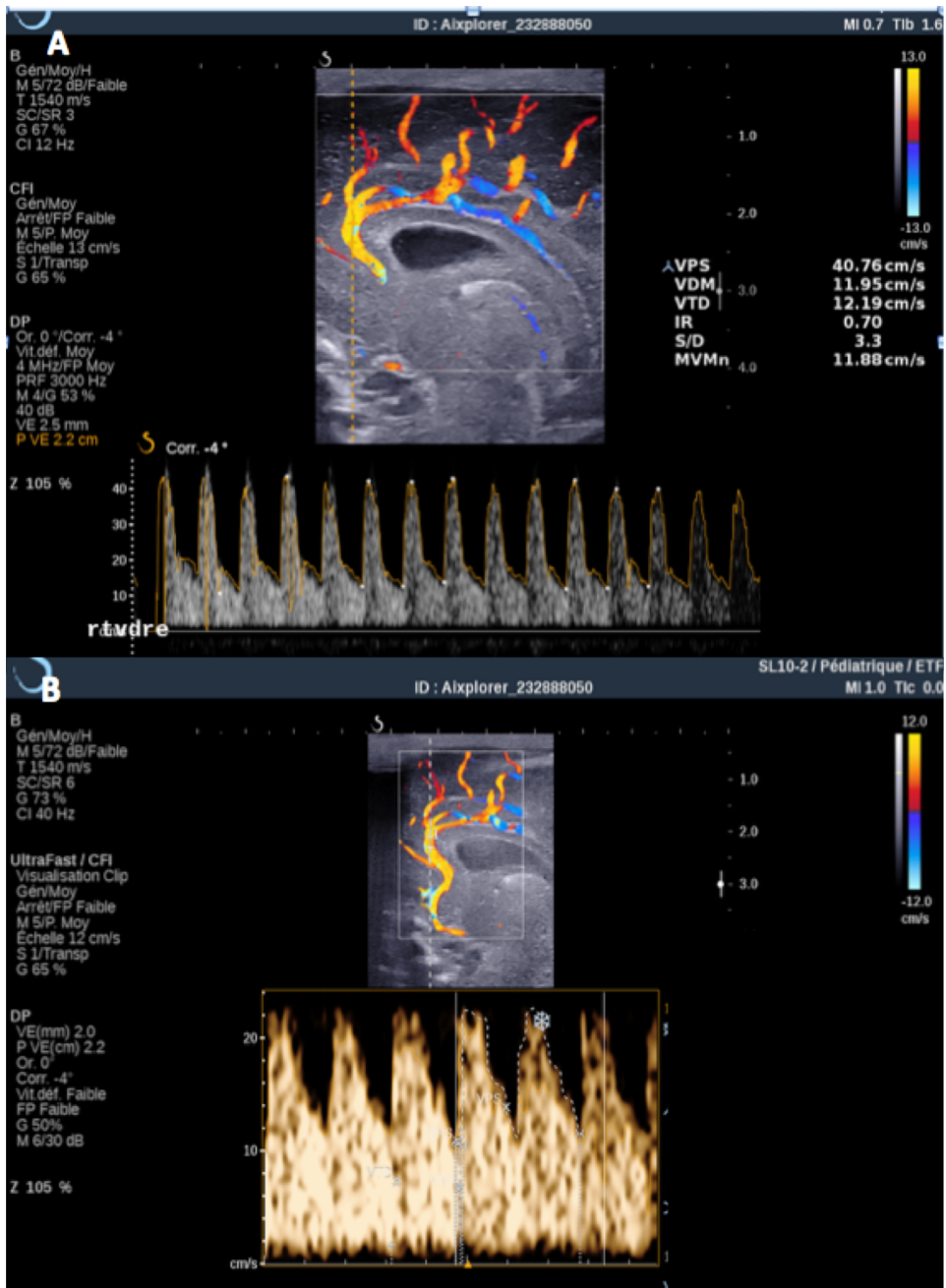


Figure 3.

Images échographique et spectrogrammes de l'artère cérébrale antérieure gauche en doppler pulsé conventionnel (A) et en doppler Ultrafast® (B) chez un nouveau né prématuré de 25 SA à J0 de vie.

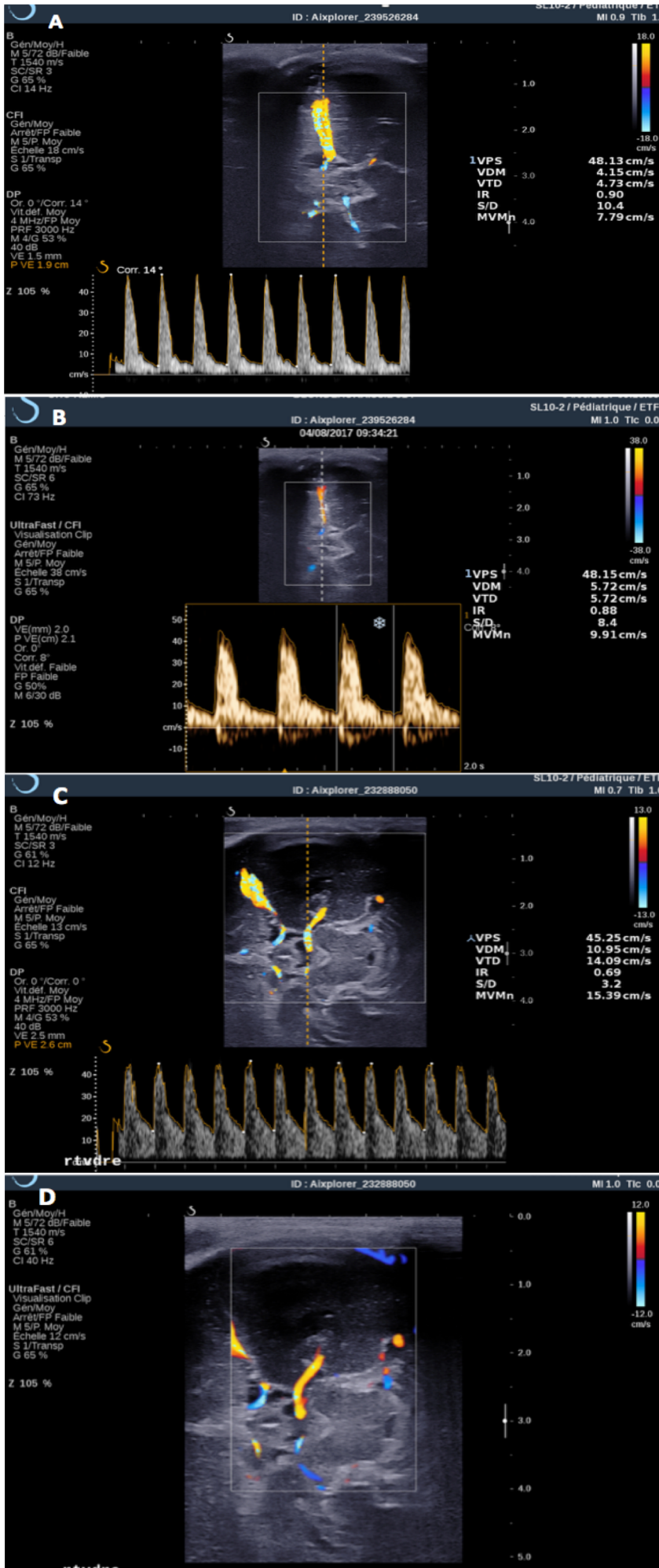


Figure 4.

Images échographiques et spectrogrammes de l'artère cérébrale moyenne gauche en doppler pulsé (A) et en Ultrafast® (B) ainsi que de l'artère cérébrale postérieure gauche en doppler pulsé (C) et en doppler Ultrafast® (D) chez un prématuré à 28 SA à J14 de vie.

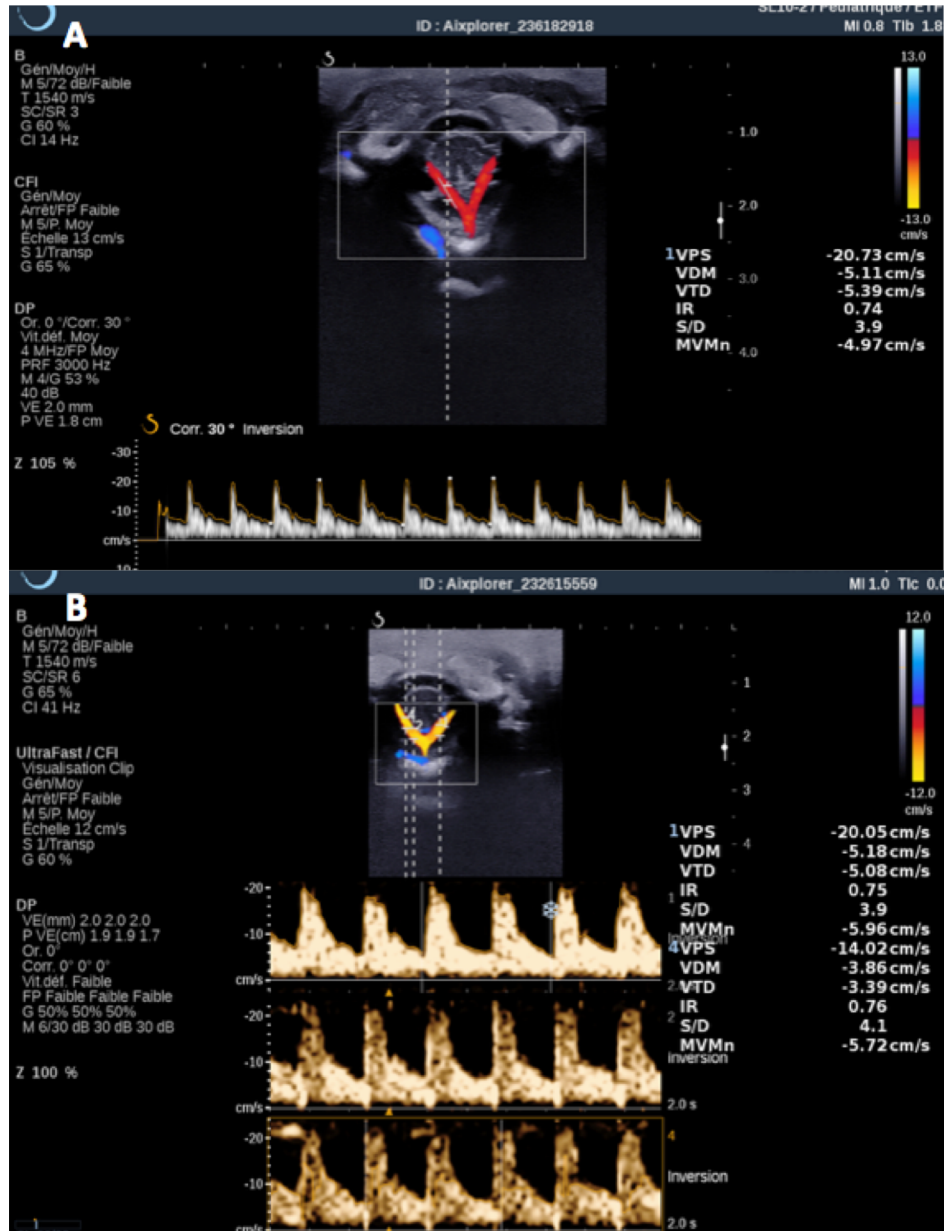


Figure 5.

Images échographiques des artères vertébrales droite et gauche et du tronc basilaire. Spectrogramme uniquement de l'artère vertébrale droite en doppler pulsé (A) et spectrogrammes simultanés des trois artères en doppler Ultrafast® (B) chez un prématuré de 28 SA à J7.

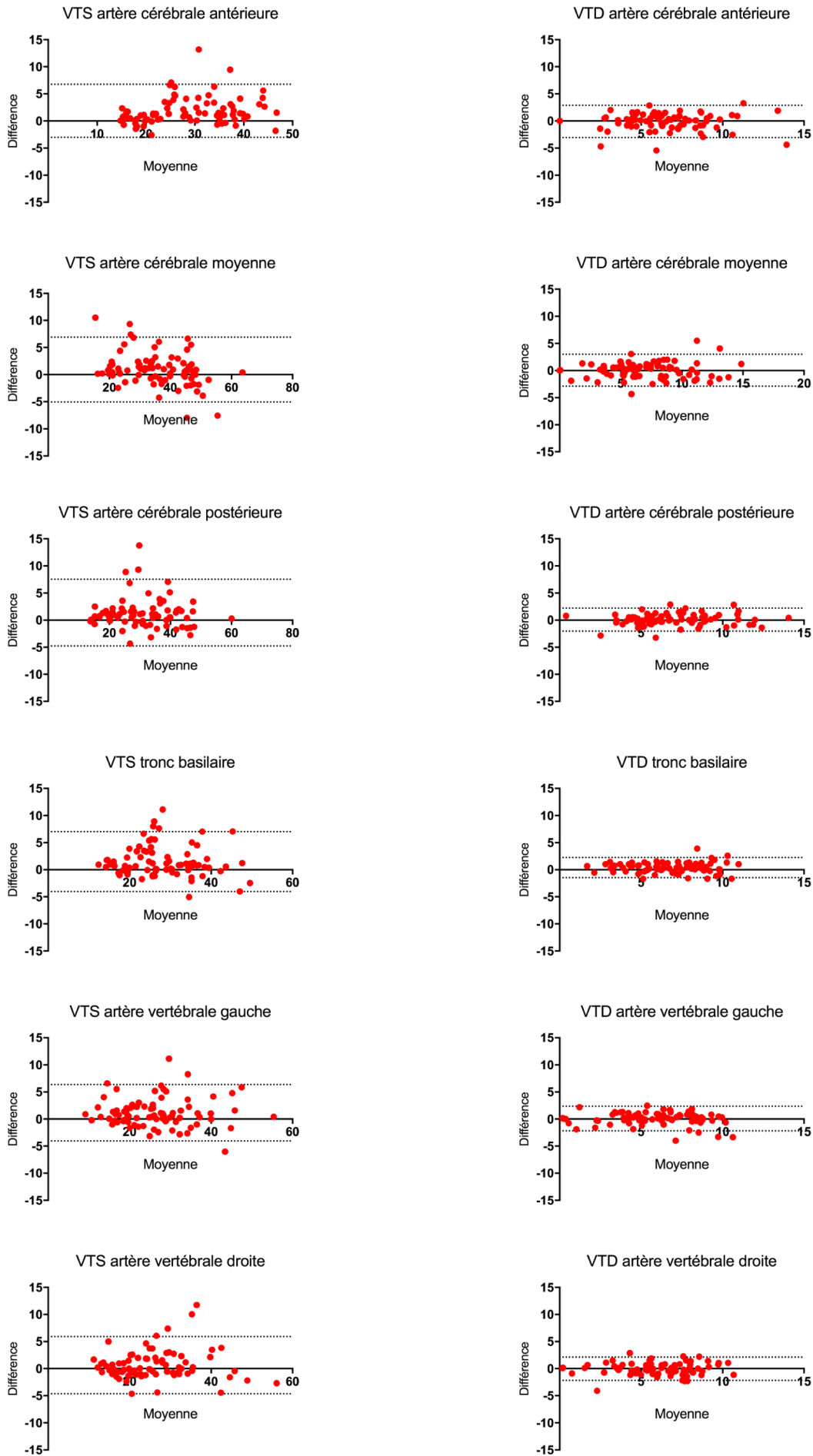


FIG. 7 Corrélation des mesures Ultrafast et Doppler Pulsé.

La différence est exprimée en fonction de la moyenne des deux mesures, selon la technique de Bland Altman. Représentation graphique pour les 6 artères étudiées, en télésystole et télédiastole

	ACA									ACM									ACP																																							
	VTS			VTD			VTS			VTD			VTS			VTD			VTS			VTD																																				
	10e	médiane	90e	10e	médiane	90e	10e	médiane	90e	10e	médiane	90e	10e	médiane	90e	10e	médiane	90e	10e	médiane	90e	10e	médiane	90e																																		
Grpe 24-26 SA	J0	14,97	15,80	19,27	0,00	3,50	7,28	19,40	22,03	24,58	2,79	4,11	5,49	14,72	17,20	20,09	3,53	5,00	7,16	Grpe 27-28 SA	J0	15,15	19,91	21,96	3,82	6,11	8,40	15,65	26,00	33,27	5,37	7,80	9,63	19,96	21,60	26,97	6,01	6,73	9,85																			
	J7	20,90	29,74	36,93	5,53	7,71	8,56	29,11	37,83	49,15	5,18	7,02	9,28	24,85	34,10	43,88	5,34	6,58	9,23		J7	23,55	29,90	37,90	5,73	7,24	8,49	25,20	31,10	49,50	5,16	7,57	9,20	27,29	36,40	43,52	5,43	7,07	11,15																			
	J14	25,63	32,02	41,16	4,02	6,35	8,72	32,86	36,95	43,62	0,94	7,15	9,52	28,91	37,46	47,81	3,86	5,80	9,05		J14	32,12	38,00	41,44	5,05	5,36	5,72	34,64	39,80	44,26	5,92	7,00	7,19	35,78	39,50	44,89	6,71	8,50	11,48																			
Grpe 29-30 SA	J0	14,90	18,64	21,20	4,44	5,82	8,72	17,27	21,32	27,10	4,33	5,51	9,22	13,96	18,90	23,49	4,07	6,39	9,04	Grpe 31-32 SA	J0	20,72	23,66	27,77	4,00	4,55	7,88	25,33	29,67	44,72	2,57	6,78	10,05	20,17	23,02	30,30	2,36	5,31	8,21	J7	25,25	29,89	36,11	5,97	7,65	8,54	35,32	46,00	48,83	4,97	9,50	13,82	26,99	33,20	39,02	4,74	8,29	9,96
	J7	33,41	37,20	41,52	2,35	7,91	9,53	40,27	48,15	55,52	2,93	6,85	11,43	28,14	39,27	51,08	5,24	6,70	12,26		J14	33,41	37,20	41,52	2,35	7,91	9,53	40,27	48,15	55,52	2,93	6,85	11,43	28,14	39,27	51,08	5,24	6,70	12,26																			
	J14	26,46	40,14	41,47	5,29	8,10	14,00	40,21	43,13	47,25	6,50	8,84	11,83	31,97	37,47	45,87	5,54	6,63	7,85		J0	20,72	23,66	27,77	4,00	4,55	7,88	25,33	29,67	44,72	2,57	6,78	10,05	20,17	23,02	30,30	2,36	5,31	8,21																			
Grpe 27-28 SA	J0	22,56	30,84	35,23	4,15	6,50	10,79	35,50	40,03	52,03	6,49	6,87	12,04	27,89	34,04	36,27	6,06	7,92	9,94	Grpe 29-30 SA	J7	22,07	30,70	39,89	4,33	6,95	9,08	17,53	27,60	35,07	3,64	7,22	8,68	20,26	23,54	39,78	5,18	6,19	7,78	J0	15,03	19,28	22,91	5,29	6,22	7,87	10,62	19,10	25,82	2,93	5,49	8,94	15,93	18,66	23,01	4,68	6,80	9,45
	J7	30,43	34,90	36,91	5,73	7,45	8,81	22,01	29,80	34,28	4,22	5,64	8,69	21,05	25,65	39,82	5,34	5,83	7,78		J7	25,19	33,15	37,33	6,45	8,30	10,14	21,98	29,60	48,05	6,86	9,00	11,04	19,48	29,25	36,39	5,02	6,14	9,11																			
	J14	20,54	36,23	42,80	2,34	6,62	9,24	27,28	36,98	45,98	4,64	6,88	8,94	21,04	31,98	50,56	3,63	7,34	8,18		J14	20,03	22,45	27,09	4,36	6,49	7,84	13,60	20,63	24,50	0,11	6,01	7,04	19,14	23,50	28,40	4,05	6,73	8,03																			
Grpe 31-32 SA	J0	24,35	32,15	34,75	5,15	7,53	10,23	18,35	30,16	35,60	3,59	6,98	9,68	19,03	28,14	29,77	5,31	6,04	8,19	Grpe 27-28 SA	J7	27,34	34,30	44,84	5,22	7,25	9,35	22,76	29,90	43,66	3,48	6,34	9,67	30,73	33,12	36,95	6,04	7,00	8,48	J0	20,03	22,45	27,09	4,36	6,49	7,84	13,60	20,63	24,50	0,11	6,01	7,04	19,14	23,50	28,40	4,05	6,73	8,03
	J7	24,35	32,15	34,75	5,15	7,53	10,23	18,35	30,16	35,60	3,59	6,98	9,68	19,03	28,14	29,77	5,31	6,04	8,19		J7	24,35	32,15	34,75	5,15	7,53	10,23	18,35	30,16	35,60	3,59	6,98	9,68	19,03	28,14	29,77	5,31	6,04	8,19																			
	J14	27,34	34,30	44,84	5,22	7,25	9,35	22,76	29,90	43,66	3,48	6,34	9,67	30,73	33,12	36,95	6,04	7,00	8,48		J14	27,34	34,30	44,84	5,22	7,25	9,35	22,76	29,90	43,66	3,48	6,34	9,67	30,73	33,12	36,95	6,04	7,00	8,48																			

Tableau 2. Normes de vélocimétrie

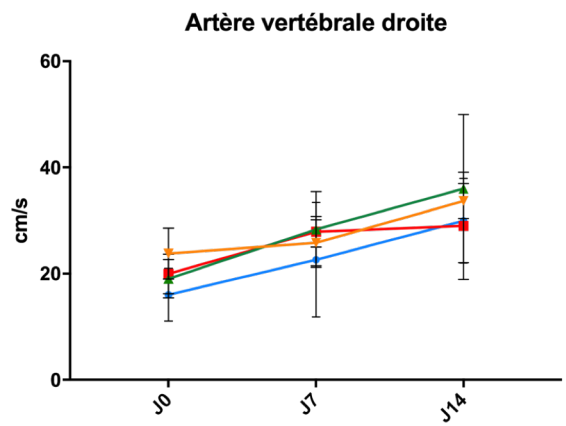
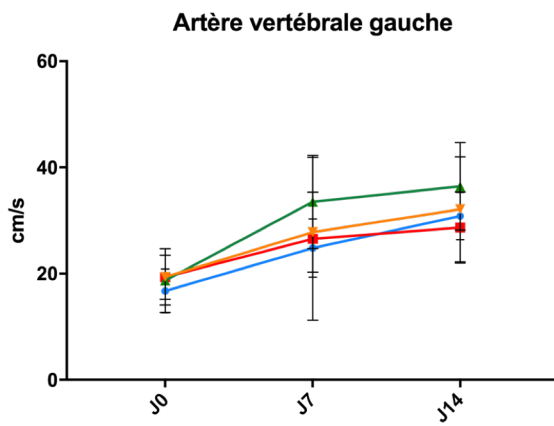
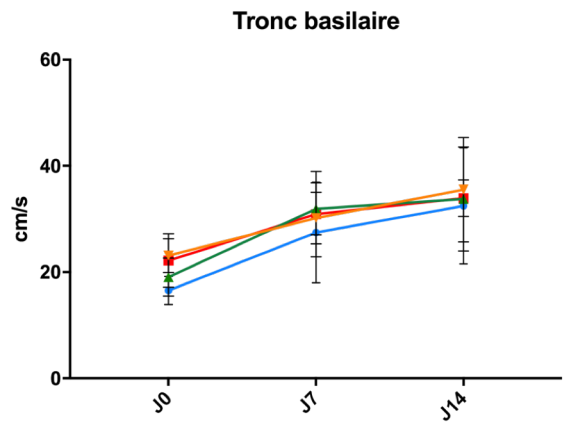
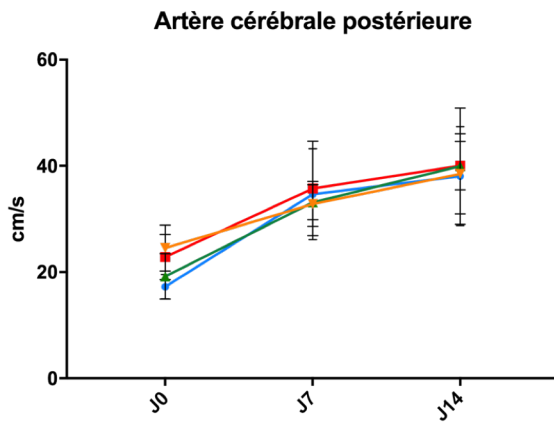
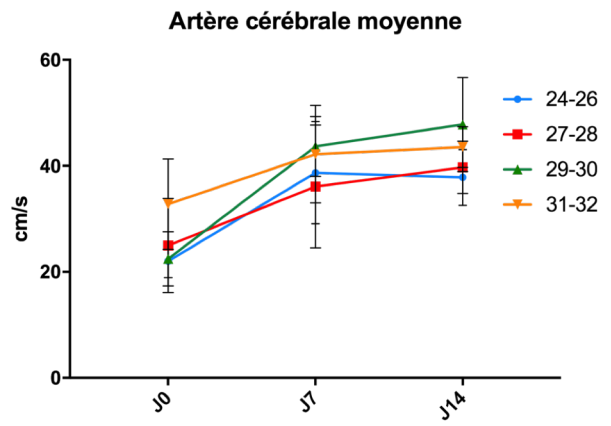
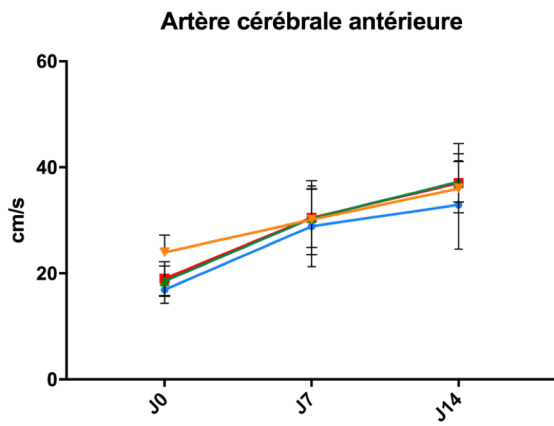


Figure 8
Evolution des vélocités télé-systoliques de J0 à J14

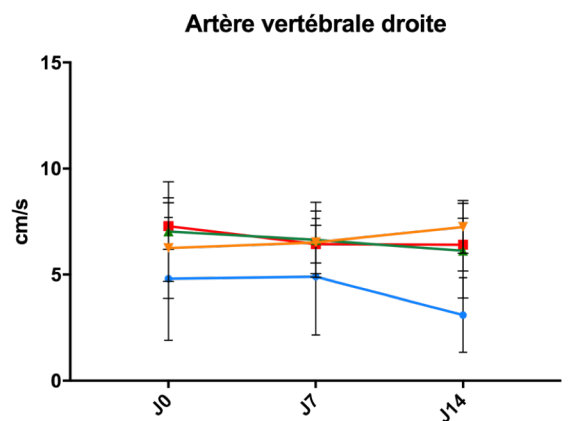
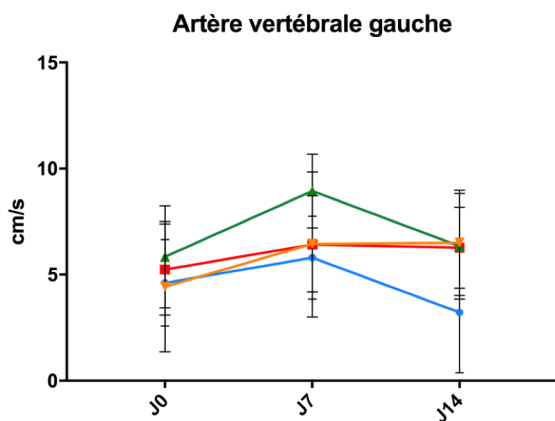
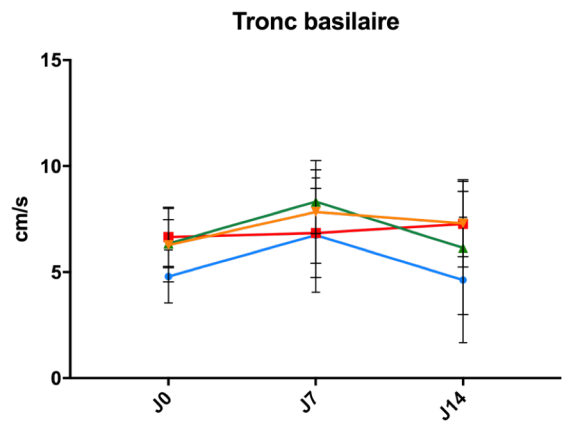
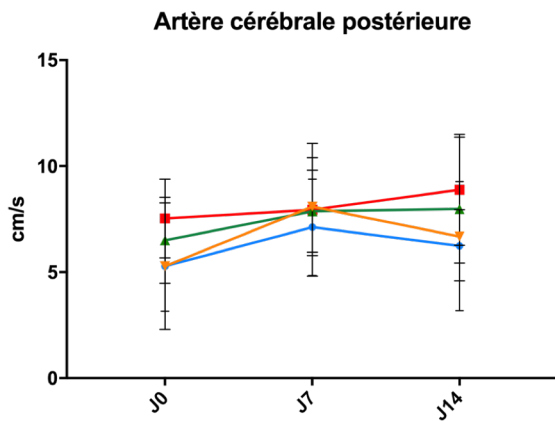
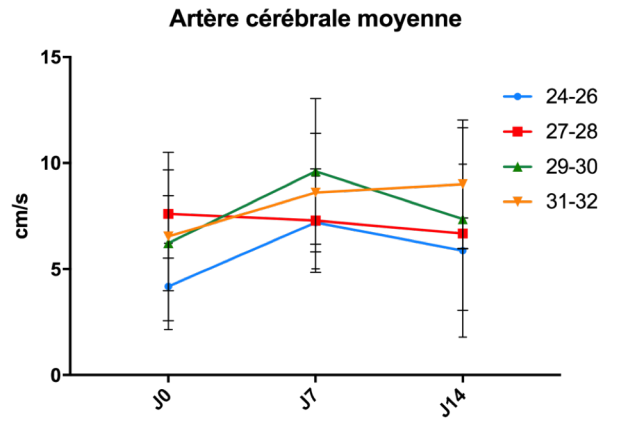
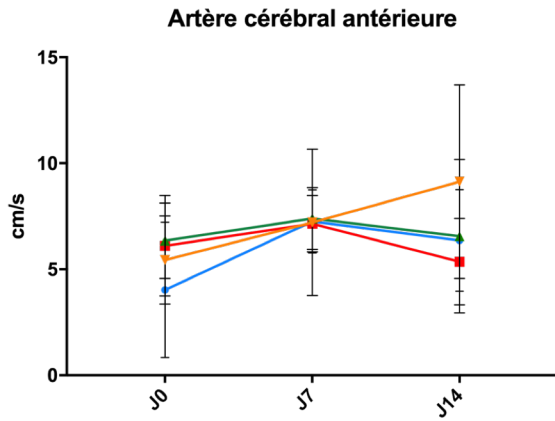


Figure 9
Evolution des vélocités télé-diastoliques de J0 à J14

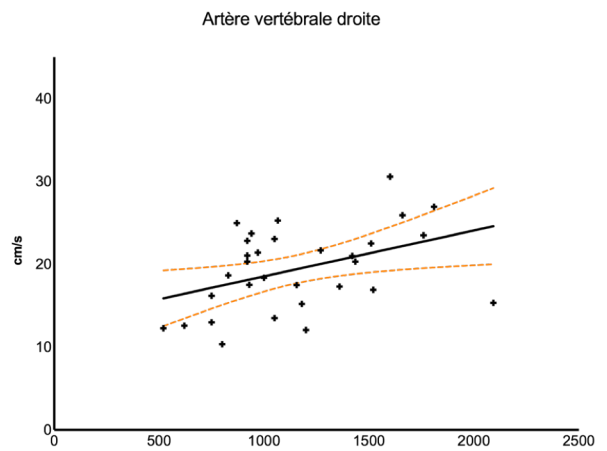
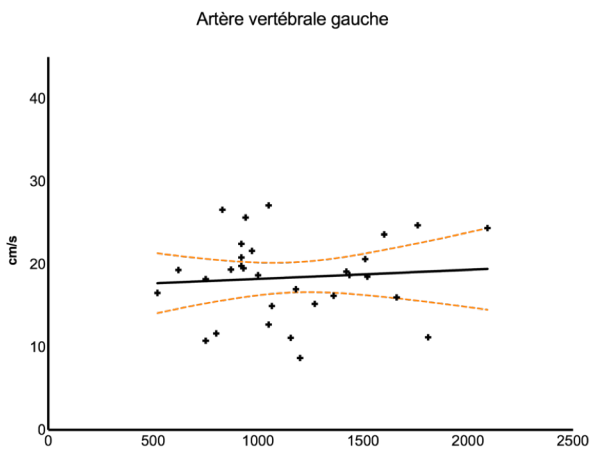
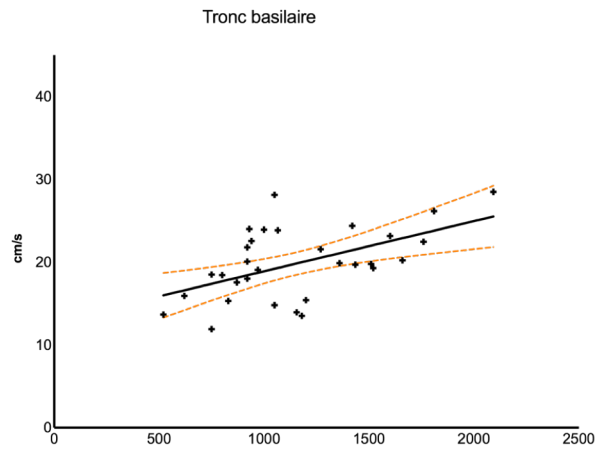
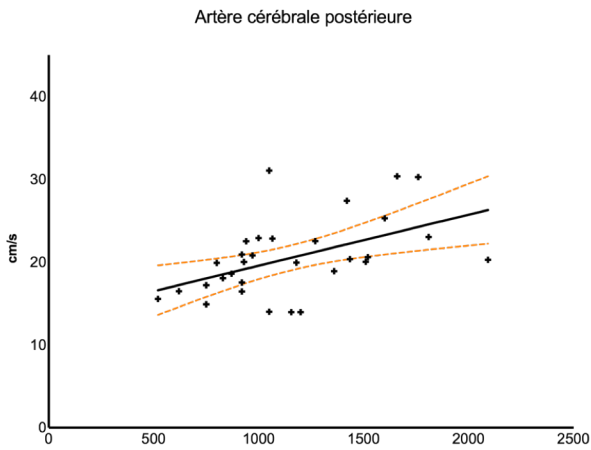
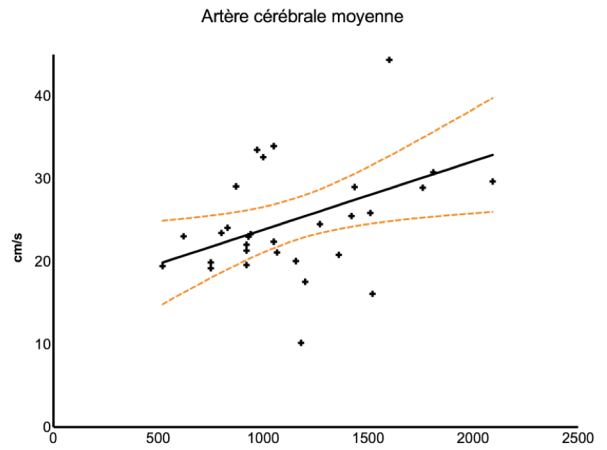
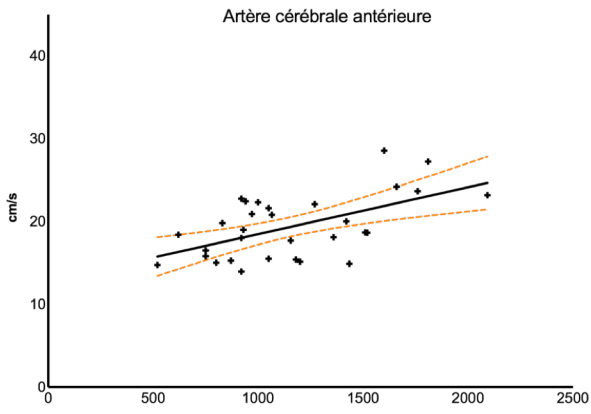


Figure 10
 Vélocities systoliques à J0 sur les 6 artères d'intérêt.
 Régression en fonction du poids

Review

Advanced Brain Imaging in Preterm Infants: A Narrative Review of Microstructural and Connectomic Disruption

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Abstract: Preterm birth disrupts the in utero environment, preventing the brain from fully developing, thereby causing later cognitive and behavioral disorders. Such cerebral alteration occurs beneath an anatomical scale, and is therefore undetectable by conventional imagery. Prematurity impairs the microstructure and thus the histological process responsible for the maturation, including the myelination. Cerebral MRI diffusion tensor imaging sequences, based on water's motion into the brain, allows a representation of this maturation process. Similarly, the brain's connections become disorganized. The connectome gathers structural and anatomical white matter fibers, as well as functional networks referring to remote brain regions connected one over another. Structural and functional connectivity is illustrated by tractography and functional MRI, respectively. Their organizations consist of core nodes connected by edges. This basic distribution is already established in the fetal brain. It evolves greatly over time but is compromised by prematurity. Finally, cerebral plasticity is nurtured by a lifetime experience at microstructural and macrostructural scales. A preterm birth causes a negative and early disruption, though it can be partly mitigated by positive stimuli based on developmental neonatal care.

Keywords: preterm infants; microstructure; connectivity; MRI



Citation: Vo Van, P.; Alison, M.; Morel, B.; Beck, J.; Bednarek, N.; Hertz-Pannier, L.; Loron, G. Advanced Brain Imaging in Preterm Infants: A Narrative Review of Microstructural and Connectomic Disruption. *Children* **2022**, *9*, 356. <https://doi.org/10.3390/children9030356>

Academic Editor: Gire Catherine

Received: 29 January 2022

Accepted: 2 March 2022

Published: 4 March 2022

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1. Introduction

Over the past decades, a shift has been taking place in the spectrum of impairments sustained by preterm born infants (PT). Medical progress has led to a decrease of mortality, as well as severe cerebral palsy [1–4]. Meanwhile, childhood follow-up studies reveal altered cognitive outcomes, with high prevalence of executive function and neuropsychiatric disorders [4,5]. Schooling is frequently impaired, and requires special educational support. Moreover, social integration and quality of life may be affected [6].

The understanding of those disabilities has greatly benefited from brain magnetic resonance imaging (MRI). Cerebral ultrasound remains an optimal investigation in daily practice to detect intraventricular hemorrhages and cystic leukomalacia [7]. However, MRI allows a more accurate investigation of the brain. It corroborates the emergence of mild to moderate motor and cognitive disorders to the histological cerebral lesions and the concept of encephalopathy of prematurity described by J Volpe [8]. Indeed, neonatal

MRI studies in the early 2000s have identified white matter (WM) signal abnormalities as nodular periventricular leukomalacia in favor of gliosis lesions and predictive of neurodevelopmental outcomes [1,9,10]. Therefore, cerebral MRI at term equivalent age has been suggested to become part of a routine imaging protocol in PT [11]. Furthermore, advanced MRI techniques have brought great insights in PT brain development. They can explore the brain's microstructure and connectivity [12,13]. At a microstructure scale, diffusion tensor imaging (DTI) provides maturational information, especially the axonal myelination. At a macroscopic scale, tractography maps physical WM fibers connections, whereas functional MRI assesses neuronal networks beyond anatomical structures. The former is defined as structural connectivity and the latter as functional connectivity. Micro and macro structures are closely related to determine cerebral organization.

Prematurity leads to increase susceptibility of the brain at a microstructural and connectomic scales. Furthermore, such disruption is exacerbated by multifactorial aggressions (inflammation, pain, etc.) that stray the brain from its initial developmental trajectory [14,15]. However, it can be counter-balanced and even reversed by neonatal interventions based on developmental care [16]. The cerebral remodeling due to external stimuli defines the concept of plasticity.

This narrative review seeks to describe normal fetal brain development, the principles of MRI techniques to assess microstructure and connectivity, and how these techniques can help to better understand the impaired brain development in PT.

2. Materials and Methods

Keywords or MeSH terms were combined in a PubMed search for this review. A first request ("Magnetic Resonance Imaging" [Title/Abstract] and "brain injur *" [Title/Abstract] and "premature *" [Title/Abstract]) or (("Magnetic Resonance Imaging" [MeSH Terms] and "Brain Injuries" [MeSH Terms] and ("Premature Birth" [MeSH Terms] or "infant, premature" [MeSH Terms] or "infant, extremely premature" [MeSH Terms])) identified 214 relevant articles. An additional 17 articles were identified by this second query: ("Magnetic Resonance Imaging" [Title/Abstract] and "brain injur *" [Title/Abstract] and "premature *" [Title/Abstract]) or (("Magnetic Resonance Imaging" [MeSH Terms] and "Brain Injuries" [MeSH Terms] and ("Premature Birth" [MeSH Terms] or "infant, premature" [MeSH Terms] or "infant, extremely premature" [MeSH Terms]) and ("plasti *" [All Fields] or "connective *" [All Fields])). Most relevant or most cited articles were selected and so some studies were carefully excluded.

As a narrative review, authors did not deem it necessary to follow PRISMA guidance.

3. Normal Cerebral Development and Neuropathology of Preterm Brain Injuries

3.1. Brain Development during the Third Trimester of Gestation

Many cellular processes take place during the third trimester of gestation—late neurogenesis up to the 25th gestational week, late neuronal migration, gliogenesis, synaptogenesis, myelination, and selective apoptosis [17]. Furthermore, a transient cortex called subplate is involved in the neurons' guidance during their migration [18]. From the ninth gestational week to the first year of age, subplate and cortex successively exercise the same functional role, connecting one over another cortical areas, deep grey matter (GM) and the spine. The functional transition from the subplate to the cortex is a crucial process, during which any upset could trigger many neurodevelopmental disorders [19]. Subsequently, thalamo-cortical circuits arise, involving the motor and sensory cortex, followed by the associative cortex. As early as mid-gestation, cortico-cortical and thalamocortical fibers undergo a synaptogenetic process in the cortical subplate. Eventually, the subplate vanishes by apoptosis after transferring the first loops to the cortex.

These processes are partly genetically determined and partly dependent on sensorial input and experience [20].

3.2. Prematurity: High Risk of Brain Injuries

Several types of brain damages have been identified in PT [8].

Large parenchymal defects, caused by infarction and hemorrhage, are usually associated with intra-ventricular hemorrhages. Those damages are mostly encountered in most immature and unstable infants. Approximately half of those children are exposed to serious neurologic adverse outcomes (cerebral palsy, lower IQ) or death [21,22]. On the contrary, parenchymal infarctions of Volpe, triggered by a venous thrombosis, display more variable outcomes depending on their size and location [23].

The periventricular WM constitutes a highly vulnerable area in PT. Oligodendrocyte precursors and mitochondria are particularly sensitive to insults—inflammation, anoxo-ischaemia, deficiency in neurotrophic and growth factors—leading to a myelination disruption [24,25]. Periventricular leukomalacia used to be normally described as large, cystic necrosis associated with surrounding myelination disorder. However, cystic forms account nowadays for less than 1% of WM injuries, usually resulting in cerebral palsy [26]. Since the 90s, along with changes in neonatal practices and generalization of conventional MRI, cystic lesions have been eclipsed by multifocal WM lesions involving nodular leukomalacia in favor of a gliosis process [1,9,10].

Moreover, besides WM lesions considered as “qualitative” injuries, “quantitative” impairments have been described, suggesting that in utero sequence, leading to premature birth, impacts the whole process of cerebral maturation. Without overt lesions, brain metrics, measured in two dimensions or in volume after segmentation, are significantly decreased in infants born preterm, compared to control children, indicative of an impaired growth [22].

The cellular loss and WM injuries are entangled with diffuse and distant alterations in neuronal organization involving the cortex, the subplate, and thalami, defining thus the global concept of encephalopathy of prematurity [8]. It is a complex amalgam of primary destructive disease and secondary with maturational, and trophic neuronal or axonal, disturbances. Indeed, the shift towards an ex utero environment influences many mechanisms of the developing brain involving the synaptogenesis, synapse pruning, cortical organization, rise of thalamo–cortical and cortico–cortical circuitry, and programmed cell death [27–29]. In this regard, plasticity defines a process of cerebral remodeling to adapt to its environment or interventions. DeMater and al. introduced the concept of “double edged sword” [30]. On the one hand, neonatal interventions such as developmental care constitute positive stimuli and potential neuroprotective factors that enhance resilience of the brain [16,31,32]. On the other hand, noxious events (inflammation, lack of natural sensorial input, nociception, pro-apoptotic drugs . . .) cause a maladaptive plasticity of the brain, which is hence discarded from its physiological route [14,15,33–35].

4. MRI Methods: A Multiparametric Approach of Brain Maturation, Structure, and Function

4.1. Conventional MRI

The signal intensity of the WM on conventional sequences follows the maturation of the brain, as the contrast lies on water and fat content in the brain’s structures. Compared to adults, infants’ conventional MRI sequences show reversed signal intensities in WM and GM (from 0 to 6 months old). In T1-weighted images WM intensity appears lower than the GM, and vice versa in T2-weighted images [36]. As the cerebral maturation evolves with a decrease of water and enhances the myelination process, signal intensities change over time.

However, at term, conventional MRI already features key markers of myelination. The myelination of the cortico–spinal fascicles may be followed as a high signal in T1-weighted sequences from the central sulcus to their projections towards the midbrain. The presence of a physiological signal in the posterior limb of the internal capsule (PLIC) is critical to confirm a myelination process [37]. Apart from an impaired myelination in PT, MRI can also identify WM injuries. Cystic periventricular leukomalacia is well diagnosed by both ultrasound and MRI. On the contrary, punctuate WM lesions representative of gliosis are only delineated by MRI scans as ischemic signals and less frequently hemorrhagic [11].

Finally, diffusive and excessive signal intensity of the WM may also be described in T2-weighted images. No histological substrate has yet been found.

In routine, the assessment of brain maturation on T1 and T2 sequences remains qualitative, and hence dependent on a radiological expertise. Quantitative T1 and T2 signals have been studied to measure brain's maturation [38]. However, they require additional sequences and regional reference values [36].

4.2. Diffusion Weighted MRI

4.2.1. Diffusion Weighted MRI as a Probe of Microstructural Assessment of WM and GM

Diffusion weighted MRI technique measures Brownian motion of water molecules. In brain tissue, the motion of water is not random, as it is constrained by structural obstacles, such as axonal membranes. Therefore, water molecules diffuse more freely along the direction of axonal fascicle. This directional dependence of water molecules movement is termed anisotropy. For DTI acquisition, diffusion gradients are applied in more than six directions to use a tensor diffusion model. For each 3D pixel, called a voxel, several quantitative parameters can be calculated from this tensor model: fractional anisotropy (FA) (overall directionality), mean diffusivity (MD), axial diffusivity (diffusivity along the main tensor axis), and radial diffusivity (diffusivity perpendicular to the main axis) can be derived from the tensor model (Figure 1) [39]. These parameters quantitatively assess the water diffusion properties in a voxel reflecting brain microstructure including WM and GM maturation. Along with maturation, they follow cellular milestones. For instance, FA increases in the WM, together with oligodendrocyte extension, glial cell proliferation, and pre-oligodendrocyte ensheathment [36,39]. On the contrary, FA decreases in the cortex. Immature cortical plate is initially anisotropic due to radial orientation of glia fibers and apical dendrites, and becomes isotropic at term equivalent age due to dendritic arborization [36].

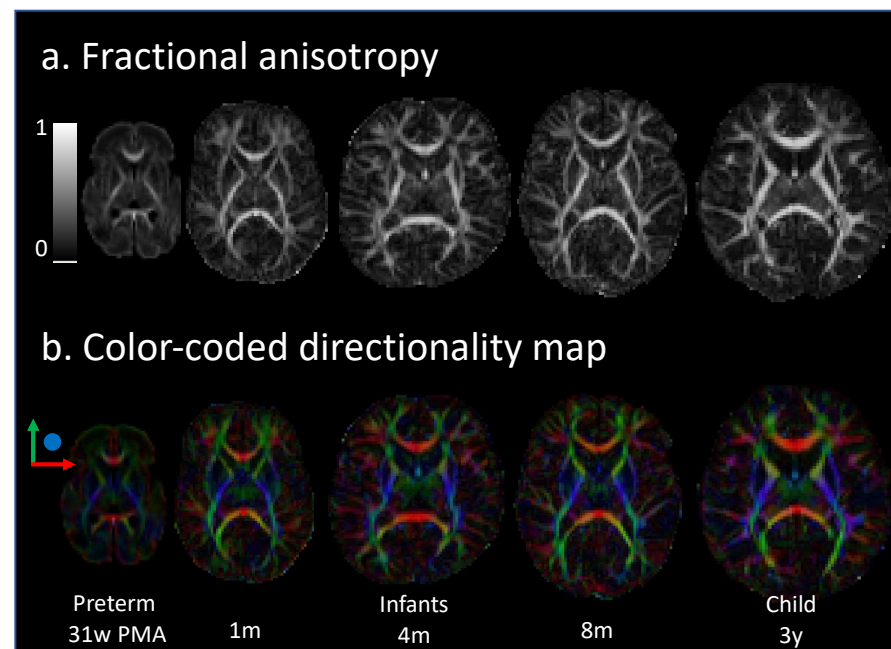


Figure 1. Evolution of DTI maps during development. Fractional anisotropy (a), and color-coded directionality (b) maps are illustrated according to the age—31 weeks of postmenstrual age, at 1, 4, and 8 months of age, and 3 years old. Adapted from ref. [36].

4.2.2. DWI Tractography and Structural Connectivity

Using DTI, directionality maps showing the main direction of the diffusion tensor for each voxel can be modeled, demonstrating the organization of main WM bundles.

Different 3D reconstruction tools can be used to assess the main direction of WM bundles in 3D. However, these tools, which are based on simple DTI considering a single fiber population per voxel, must be subject to caution due to the occurrence of false negatives (premature termination of a tract) or false positives (switch of another bundle crossing the same voxel) [40,41]. More complex tools can be used to deal with crossing fibers, but they require the acquisition of longer DTI sequences.

Structural connectivity refers to networks that anatomically interconnect brain regions. It is typically measured in vivo in humans using diffusion weighted imaging tractography by combining 3D reconstruction of fiber bundles and quantification of microstructural characteristics of those bundles using diffusion metrics (FA, MD, radial, and longitudinal diffusivities) (Figure 2).

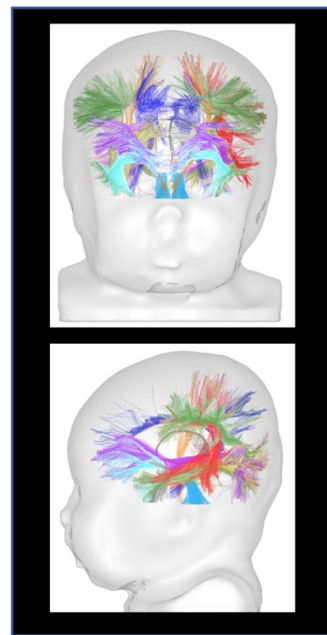


Figure 2. Tractography of WM bundles in a 1-month-old infant. The trajectory of the main WM bundles (projections, callosal tracts, limbic, and associative bundles) can be generated. Adapted from ref. [36].

4.3. Functional MRI, Functional Connectivity

Functional MRI aims to describe brain activity based on the hemodynamic response following neuronal activity using blood oxygenation level-dependent (BOLD) sequences. The local imbalance between oxygenated and deoxygenated hemoglobin during neuronal firing generates a local magnetic field change that creates a contrast in MRI [42]. Functional MRI is therefore used to probe brain activity during specific cognitive or sensorimotor tasks. In addition, it has been shown that synchronized low-frequency fluctuations of BOLD signal can be identified at rest in remote brain regions belonging to the same network (resting state functional MRI). This is the basis of the study of functional connectivity that reflects the functional architecture, i.e., the association of brain regions within long distance networks, as well as the relationships between different networks. Hence, networks identified in resting state functional MRI (rs fMRI) are named resting state networks (RSN). Resting state MRI is suitable for newborns imaging studies as it does not require patient cooperation, and can be performed during sleep.

Finally, older children could benefit from task-based MRI. It explores the effective connectivity, and may bring great insights to further understand the mechanisms of plasticity. Effective connectivity assesses the causal influences that neural units exert one over another [43]. It reflects the functional connectivity during a dynamic task.

4.4. Brain Connectome

Interestingly, while there is a large overlap between structural and functional connectivity, there is no strict correspondence between them.

Historically neuroscience stated the association between a brain region and a specific function. In other words, brain organization is based on the segregation of brain regions with a task specifically linked to an anatomical region.

In addition, more recent approaches highlighted that brain function relies on large scale functional networks composed of interactive regions, sometimes remote, revealing an integrative organization.

Brain network can be modeled accordingly following the graph theory, including an ensemble of neuronal elements termed as “nodes”, connected one to another by “edges or links”. Ideally, nodes should represent brain regions [12,44]. Links may correlate to WM tracts (structural connectivity) or to a functional circuitry (functional connectivity). Different metrics can be used to define the network organization and its general geometry or topology.

A “segregation organization” is opposed to a “network integration organization” (Figure 3). In a “network segregation”, one module contains several nodes densely linked one to another, but sparsely connected to other nodes belonging to a different community. It reflects a potential specialized function of the network. On the contrary, a network integration demonstrates a competence to travel information through different brain regions (nodes) [45].

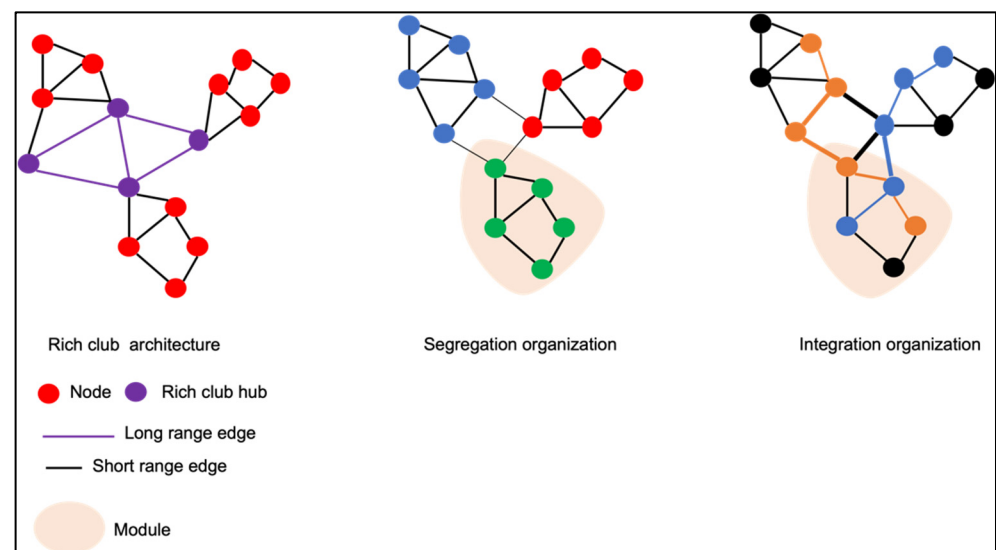


Figure 3. Illustration of a cerebral network according to the graph theory: nodes are connected one over another by edges. Nodes which play a significant role in exchanging information are hubs. Well-interconnected hubs belong to a rich club. In a segregation organization, modules represent networks with dense connections between nodes within the same module but sparse connections between nodes of other modules. In an integration organization, disparate brain modules are well-interconnected.

5. MRI Studies of Early Typical Brain Maturation, in the Third Trimester of Gestation

5.1. Maturation of WM

Anisotropy does not exclusively reflect a myelination process. It also relates to the development of oligodendrocytes and axonal density. A total of three major steps occur during the maturation of the WM [39]. First, fibers inside a bundle undergo a fasciculation phase, where oligodendrocytes line up. Second, these oligodendrocytes proliferate during a pre-myelination stage. Meanwhile, water concentration decreases as membranes densify. Finally, the myelination ensheathment of the axon takes place with decreased membrane permeability and extracellular distance between membranes. Together with the

maturation, anisotropy (FA) increases, whereas mean and transverse diffusivities decrease. Longitudinal diffusivity first increases and then decreases.

Based on histological explorations, myelination follows a central to peripheral and posterior to anterior gradient up to three years old [46]. Diffusion tensor imaging studies of in utero fetuses and PT have confirmed such time course. Between 28 to 43 weeks of gestational age, WM in cerebral peduncles, internal capsule and commissural tracts of corpus callosum (CC) were the first to emerge [45]. After birth, PT and infants' models, designed by Dubois and al., described how myelination successively progressed in the first months— (1) motor and somatosensory pathways of the cortico–spinal tract (CST), (2) spinothalamic tract and fornix, (3) visual tract of the optic radiations, arcuate and inferior longitudinal fascicles, and (4) anterior limb of the internal capsule and the cingulum [47].

Maturation is asynchronous, but also asymmetric. For instance, speech processing is related to the left perisylvian region. Moreover, human species are distinguished by a lateralized hand preference. Such asymmetry is already set early in life. As early as the first three months of life, the auditory areas of the left hemisphere have already showed a predisposition for the language processing [48]. Furthermore, at a microstructural scale, a leftward asymmetry in the arcuate fasciculus and CST was demonstrated with a higher FA compared to the contralateral side, as soon as one month of age [49]. Those two bundles are involved in the language and sensory-motor networks. However the correlation between the asymmetry of motor tracts and handedness remains debated. Moreover, correlation between structure and function is not systematic and remains one major challenge in neuroimaging of cerebral connectome studies.

5.2. Maturation of the Cortex

The cortex matures simultaneously with the development of its corresponding WM as shown by Smyser et al. [50]. On conventional MRI sequences, rolandic sulcus and CST as well as the thalamostriate pathways are easily visible at term. However cortical microstructure is more complex to analyze. It is constrained by the development of dendritic arborization, synaptogenesis, and myelination of intracortical WM. The cortical thickness and the complex cortical folding pattern also render the evaluation difficult. In DTI, GM maturation was characterized by a decrease of MD and FA as opposed to WM [51]. The diminution of MD was due to the increasing cellular density and complexity, whereas the maturation of dendritic architecture and neurite outgrowth led to a decreased FA.

Furthermore, other quantitative MRI modalities have confirmed DTI and postmortem studies [52]. Primary sensorimotor regions myelinated earlier followed by the primary visual cortex around the calcarine fissure, primary auditory cortex, and somatosensory cortex [51,52]. Such hierarchy remains over the infancy period.

5.3. Maturation of Structural and Functional Connectivity

Structural connectivity has been studied in vivo as early as 27 weeks of post menstrual age [53]. The vast majority of the large pathways present in an adult connectome was also identified in neonates [54]. In term of topology, they were already exhibiting, before term, a small-world architecture, characteristic of the adult organization. In such architecture, networks are clustered as opposed to random, which enables to process the information more efficiently. Besides, hubs, i.e., highly connected nodes, were described in the cingulate cortex, superior frontal and parietal regions, and left precentral and post gyrus. Furthermore, brain regions encountered a gradient in maturation [55]. Between 27 and 45 weeks of postmenstrual age, connections between primary sensorimotor, occipital, and frontal cortex featured the highest rate growth, especially short connections within the same hemisphere [51,53,56]. Eventually, during the prenatal stage, the structural network would evolve from a segregation of locally efficient groups of regions to an integrative organization with the development of long-range connections [56,57].

It is assumed that functional connectivity leans on an effective structural connectivity, even if it is not exactly correlated to it. Resting state networks were detectable by the

third trimester, but rather at an immature state compared to structural connectivity [56]. Primary networks, such as the sensorimotor, visual, and auditory networks, and thalamo-cortical connectivity could be detected in PT as early as 26 weeks of gestation [50,58]. Maps revealed a bilateral symmetry between hemispheres in favor of a qualitative homotopic functional connectivity. In other words, in early gestational age, strong connections remained inter-hemispheric, and became intra-hemispheric over time. Certain higher-order networks, such as the default mode network and salience network, were also delineated very early on. The salience network includes nodes in the amygdala, hypothalamus, ventral striatum, thalamus, and specific brainstem nuclei [59]. It is implicated in the integration of sensory, emotional, and cognitive information. Default mode network is built on the posterior cingulate cortex and ventral anterior cingulate cortex, and is activated during resting states [60].

As neonatal functional architecture greatly overlaps with the adult's connectome, it should constitute the proto-architecture that would refine along the post-natal life [54]. Graph theoretical model enabled to describe similar hubs and rich club architecture as early as 31 weeks of post menstrual age in primary motor, somatosensory, visual, and auditory regions [61]. A rich club organization described well-interconnected hubs, which has been proven to be efficient for communication within the brain. Along with gestational age, the organization evolved with more efficient local connectivity, as well as the development of long-range connections. Finally, similarly to structural connectivity the balance between a segregation and integration organization favored the former [56].

6. Brain Alterations in PT

6.1. WM Microstructural Alterations

6.1.1. Cerebral Injuries and Risk Factors of WM Alterations Disruption

Brain lesions may disrupt the underlying periventricular WM bundles [21,62–67]. A recent prospective study included PT sustaining brain injuries compared to uninjured PT and full-term infants [67]. Altered WM with reduced FA was found in the PLIC, the right cingulum, the lentiform nuclei, and the CC. Higher MD was also measured in both optic radiations and cingulum due to increased edema and lower cellularity. Interestingly, on the one hand, disruption of the WM was predominantly located in the caudate nucleus and CC in intra-ventricular hemorrhages. On the other hand, post hemorrhagic ventricular dilatation was related to alteration of the internal capsule and optic radiations [63,67]. Nodular periventricular leukomalacia was also associated with lower FA values in the PLIC, cerebral peduncles, corona radiata, and arcuate fasciculi, along the CST [62,64,65]. The load of punctuate lesions was negatively proportional with FA.

Finally, all factors causing a systemic inflammatory state such as broncho-pulmonary dysplasia, infections, necrotizing enterocolitis, and intra-uterine growth restriction, constituted risk factors to a disruption of the WM microstructure [41,66,68,69]. Number of days of parenteral nutrition and mechanical ventilation, higher social risks, and male sex also predisposed WM alteration [66,69].

6.1.2. Alteration of the WM Microstructure in PT without Apparent Brain Injuries

Even in the absence of obvious brain lesions, the preterm period, as an early ex utero life extraction, would independently cause a disruption of the cerebral microstructure. Literature is relatively vast, yielding various results. In PT, an overall alteration occurred in a wide range of WM bundles known to play a role in motor, language, reading, or mathematic skills [41,70–73]. Among all the anatomical sites, the CC was widely studied as a major inter-hemispheric track vulnerable to ischemia and inflammation, showing a reduced FA which interestingly raised the hypothesis of a less efficient inter-hemispheric transmission [41,70–73]. Moreover, Li and al. gathered studies in a meta-analysis comparing PT versus term infants through different periods of life: term equivalent age, infancy, childhood, and adolescence [70]. The decreased FA of numerous WM fascicles persisted over every period of the timeline [40,70–72]. One should bear in mind that FA values

need to be interpreted according to the age of PT. In the early infancy myelination has not occurred neither in the splenium nor in the genu of the CC. A fall of FA might then be related to a delay of the pre-myelination stage with lesser pre-oligodendroglial cells, and disorientation along the axonal axis [39].

Finally, at the cortical level, FA also declined in PT, along with the maturation and hence gestational age [51]. However, the speed of drop was reduced in PT with low gestational age. At term-corrected age, PT showed higher FA than term infants.

6.1.3. Correlation of WM Abnormalities with Neurodevelopment

The widespread disruption of WM involves numerous bundles which share multiple roles in both motor and cognitive development. Consequently, their disturbance at a microstructure scale have been related to disabilities [51,74]. Prematurity and WM lesions both acted as independent factors.

A slower maturation, i.e., a slower increase of FA during the preterm period, and a lower FA at term equivalent age in the basal ganglia, CC, and PLIC, were associated with a worse motor and cognitive outcome at 18–24 months [63,74,75]. Griffiths' developmental quotient also positively correlated with the maturation rate of FA in regions of interest placed in frontal, parietal, and occipital cortex [51].

Such link was confirmed by long-term follow-up. At seven years old higher MD, axial and radial diffusivity, in the cerebellum, and the inferior occipital region, were related with both motor and cognitive impairments [64]. Intellectual quotient and reading skills were positively associated with FA in the occipital CC at age seven [41]. Reduced FA in the CC was also linked to impaired attention performance, and specifically in orientation scores. Finally, a strong correlation was shown between a reduced FA in the left cingulum, the superior longitudinal fasciculi and attention, orientation, or alerting, performances [71].

At adolescence, the same pattern between WM disruption of those identical bundles remained in relation with poor executive function [73]. PT teenagers with mathematic difficulties had lower neurite density and lower FA in the corona radiata, internal and external capsule, and the left fronto-occipital fasciculus [76].

6.2. Abnormal Structural Connectivity in PT

6.2.1. Cerebral Injuries and Structural Connectivity

Tractography finely illustrates in 3D how WM tracts are damaged by brain lesions [12,77]. In PT with unilateral periventricular hemorrhagic parenchymal infarctions, the subsequent WM loss reduced the efferent CST volume. Moreover, those patients showed a contralateral hemiparesis as early as the first year of age. However, the afferent thalamo-cortical tract was relatively spared as it could circumvent the area of focal WM injury [77]. In some patients, the thalamo-cortical tract delineated two routes to link the thalamus to the perirolandic region, at term equivalent age. The first one connected the thalamus to the insula area, and the second one the insula to the perirolandic region. Interestingly, this double pathway was no longer found at one year, but rather consolidated into one single tract. As the CST matures rapidly especially in the early third trimester of gestation, a compensatory mechanism would be limited. By contrast, thalamo-cortical afferences still undergo a maturation process through the subplate to reach the cortical layers. It allows to take advantage of a potential plasticity and establish a compensatory route.

Moreover, Smyser and al. have represented the benefit of ventriculoperitoneal shunt in post-hemorrhagic ventricular dilatation [12]. Tractography illustrated how, in one week after the ventriculoperitoneal shunt, CST of the less injured hemisphere had grown significantly in a compensatory manner, whereas the contralateral CST which was more severely damaged remained small.

Interestingly, in parieto-occipital leukomalacia, the visual WM pathways, i.e., the optics radiations, the ventral visual stream (inferior longitudinal fascicle, and inferior fronto-occipital fasciculus) and the posterior thalamic radiations displayed an abnormal connectivity [78].

However, similarly a reorganization occurred in the fronto–striatal and fronto–limbic pathways with multiple nodes showing higher efficiency, acting like a compensation.

6.2.2. Structural Connectivity Alteration in PT without Apparent Brain Lesions

The disruption of the structural connectivity by the prematurity has coincided with a decrease of the cerebral volume and an altered microstructure [79–81]. It prevailed in the thalamo–cortical structural network associated with a reduction of the thalamic volume [82]. The structural connectivity remained impaired at term equivalent age.

In term of organization and topology, connections with a fast growth passing through the deep GM were preserved by the prematurity [51,55]. Furthermore, between 30 and 40 weeks of gestation, connections between core hubs kept proliferating in order to establish strong networks at term. Overall, PT favored a segregation organization and a rich club architecture, a core of local highly connected organization. However, its existence seems to be detrimental to the brain regions, with a slower physiological growth [51]. Indeed, local connectivity, involving the cerebellum and thalamus, was the most impacted by the degree of prematurity as they showed less efficient connections [55].

This disrupted pattern seems to persist over time [44,83]. From 1 to 3 years of age, the fronto–temporal region including the anterior centrum semi-ovale, corona radiata, and the genu of CC, would normally undergo a critical development in terms infants. Yet, it was not the case in PT who still encountered a widespread reduced connectivity [83]. Differences also remained at six years in the frontal region and the limbic system. Moreover, a rich club architecture and a segregation organization prevailed even at that age over a normally integrative pattern [44,84]. As a consequence, PT might lack the ability to exchange information between different brain regions. Interestingly, in the same cohort of six years old children, intra-uterine growth retardation was a factor of connectivity disorder.

Finally, such disruption was confirmed in young adults [81]. Networks implicated in somato-sensory functions, i.e., precentral gyrus, mid temporal area, deep GM, and cerebellum pathways, remained more organized locally than in term children keeping the same pattern as described above.

6.2.3. Structural Connectivity and Outcome Prediction

Tractography 3D modeling of damaged CST is an intuitive and educative way of predicting motor outcome. However, conventional imaging already demonstrates good specificity and sensitivity for motor prognosis [85]. One purpose of connectivity research is to correlate structural and functional networks to cognitive disorders. In this regard, studies are relatively scarce. Bayley score at two years was related to thalamocortical pathways integrity [86]. Moreover, performance IQ of WPPSI scale was significantly associated with markers of integration and segregation networks [87].

6.3. Functional Connectivity Alteration

6.3.1. Functional Connectivity Disruption by Brain Injuries, and Plasticity

As opposed to structural connectivity, the functional connectivity's disruption due to a brain lesion does not systematically reflect the damage of the WM tract itself. It may also reveal indirect consequences of WM injuries. Diffuse excessive hypersignal intensity described on conventional MRI was associated with aberrant connectivity in executive and fronto–parietal RSN, which were linked to executive functions and language processing [88]. Moreover, severe intra-ventricular hemorrhages or moderate–severe periventricular leukomalacia impacted RSN located in the thalamus, motor, auditory, visual, cortices, and the cerebellum [89]. The thalamo–cortical connectivity was also disrupted proportionally to the severity of the injury. In this regard, inter-hemispheric (long range connectivity between homotopic counterpart) and intra-hemispheric (short range connectivity within the regions of interest) connections were decreased compared to control.

Along with the altered thalamo–cortical connectivity in WM injuries, connectivity was increased in the salience pathway [61]. Interestingly, this pathway was implicated in the

“global neuronal workspace” circuit, which was responsible of neonatal consciousness and sensory process [90]. Altered thalamo–cortical connectivity might also be compensated by a higher inter-hemispheric connectivity, especially in peri-rolandic and insular regions [77]. It was demonstrated in PT who sustained less severe, peri-ventricular hemorrhagic infarctions, and leukomalacia [61,77,90]. The preservation of the inter-hemispheric connectivity has been playing a key role in plasticity to guarantee a better neurodevelopmental outcome. Interestingly, loss of inter-hemispheric functional connectivity in post hemorrhagic ventricular dilatation has been rapidly reversible after ventriculo–peritoneal shunt [12]. Finally, in some cases, functional connectivity in GM within the injured area was spared or otherwise increased [61,89]. This provided evidence of another compensatory mechanism. Functional WM disruption is a complex ensemble of direct and collateral effects, involving respectively mono-synaptic and polysynaptic connections.

6.3.2. Functional Connectivity Disruption in PT without Apparent Brain Lesions

The disruption of the functional connectivity observed in PT at term equivalent age involves the thalamo–cortical and cortico–subcortical pathways [58,91,92]. A significant reduction was demonstrated between thalamus, sensorimotor cortex, brainstem, and cerebellar vermis [58]. Moreover, connections between basal ganglia nodes and frontal cortex nodes were found to be less prominent in PT compared to term infants [91].

Furthermore, PT displayed less long-range connections [58]. Interhemispheric connections were also less robust.

In addition, PT infants showed a dense locally connected organization [93]. A core set of nodes may be identified in posterior cingulate cortex, inferior parietal, temporal lobes, and visual cortex, but featured a reduced strength compared to term infants, with a decreased connectivity between hubs, and a less functional segregation.

Finally, functional disruption persists over time [94]. At 18 and 36 months old, functional connectivity was stronger within the visual areas among PT, whereas term infants displayed a stronger connectivity between motor and both visual and frontal regions. Such dysconnectivity between visual and motor areas was confirmed in adolescence, as well as in the dorsal attention network [95]. In contrast, higher connectivity was found between the sensory motor network and the central executive and salience networks. Interestingly, the hyperconnectivity suggested several hypotheses by the authors: first, a plasticity mechanism to cope with the counterpart hypoconnectivity, or conversely a WM disruption effect, especially the lack of synaptic pruning.

6.3.3. Functional Connectivity and Outcome Prediction

The predictive value of functional disruption on neurodevelopment needs further studies. However, ex PT children and adults with cerebral palsy displayed a reduced connectivity of the motor cortex towards the bilateral paracentral lobule and the cingulate motor region, the thalamus connections to the caudate nuclei and the cingulate cortex and, between the sensori-motor and visuo-motor pathways [96]. Furthermore, describing how functional and structural connectivity may interact with each other proves instructive [97]. Compared to control, children and young adults with cerebral palsy exhibited in the whole brain a reduced efficiency at a structural scale, but also a rather intact functional network. Such results rose the hypothesis of a functional reorganization. The opposite pattern was demonstrated in the motor network as the structural connectivity was preserved whereas the functional one showed reduced global efficiency. Hence, authors have raised the hypothesis of a structural reorganization. It is difficult to conclude which mechanism is more critical for the neurodevelopment.

7. Negative and Positive Plasticity of the Brain during the Neonatal Period

As the brain undergoes a critical developmental process during the neonatal period, it is further influenced by exterior stimuli. Nowadays, neonatal therapeutic strategies are based on developmental care. They aim to minimize stressful stimuli from the environment

and promote sensory stimulating care. In this regard, negative and positive experiences impact cerebral microstructure and connectivity.

7.1. Stress and Nociceptive Stimuli

Any event that engenders an over-stimulation for the newborn would be considered stressful. Therefore, stress encompasses a large variety of stimuli. It ranges from changing the diaper to painful procedures.

Pain overstimulates vulnerable and immature neurons, through excitotoxic cellular damage with excess of calcium and glutamate release [98]. Axonal development alters subsequently. Physiologically, thalamo-cortical and limbic pathways play an important role in nociceptive encoding [99]. Indeed, thalamus conveys nociceptive signal to cortical areas, including the somatosensory and limbic cortices. As for the brainstem, the limbic system and the hypothalamus are responsible for a feedback loop that modulates the spinal dorsal horn response to an external stimulus. It allows to refine sensory inputs as tactile, thermal, or painful sensation. Such development process has been lacking during the neonatal period [14,15]. Therefore, PT have been subjected to a central sensitization to repeated painful stimuli without inhibitory feedback, which subsequently disrupted the microstructure and the connectivity. Furthermore, in early life the descending pathway regulation to the spinal activity is more likely conducive to “lower threshold” tactile stimuli. By contrast, inhibitory retro-control to “high threshold” painful input occurs later in life, in particular after the preterm period. Authors have also postulated that such a process was compromised by prematurity [14,15].

At a microstructure scale, nociceptive stimuli (skin breaks) might affect the anisotropy diffusively, including basal ganglia, thalami, optic radiations corpus callosum, and temporal lobes [33–35]. At a structural scale, pain was associated with a thalamic volume loss in concordance with an altered connectivity in thalamic and sensory motor RSN [14,15]. A decreased functional connectivity of the insula and the limbic system was also demonstrated, including connections between the right amygdala and the ipsilateral hippocampal and parahippocampal, regions [15]. Moreover, a weaker functional interhemispheric connectivity from the right temporal cortex was found.

It is worth noting that glucose suction did not have any effect positive effect on the WM disruption, and that the impaired connectivity concerned PT at an early age rather than at term corrected age [15,35].

Finally, as early as in the prenatal period, anxiety and depression of pregnant women perturbed the amygdala’s functional connectivity to the brainstem, the thalamus, and the hypothalamus with a reduced network’s strength [100]. As the amygdala connectivity is regulated through GABAergic transmission, authors have suggested that stress disrupted the migration of GABAergic progenitors and impaired synaptogenesis.

7.2. Positive Stimulation and Musicotherapy

Musicotherapy is used to relieve stress in neonatal care, and may improve a newborn’s well-being [31]. Musicotherapy promotes maturation of tracts involved in auditory and socio-emotional processing [16]. Indeed, compared to PT without any interventions, PT with musicotherapy showed higher FA in acoustic radiations (which transmit auditory information from the thalamus to the primary auditory cortex), uncinate fasciculi (which participate at emotion’s encoding), and in the external capsules.

The beneficial plasticity of microstructure matches with functional connectivity results [101]. Saliency RSN connects modules implicated in sensory perception and cognition. Musicotherapy enhances connectivity in those modules that involve the auditory, sensorimotor, superior frontal, thalamus, and precuneus networks.

Therefore, musicotherapy is a promising tool to prevent the detrimental effect of prematurity on the cerebral maturation and organization.

8. Conclusions

Premature birth deprives the brain from its physiological in utero environment, during a critical period of intense development. Prematurity is disruptive at a microstructural and connectomic scales, which constrain the brain maturation and organization. Subsequently, aberrant circuitries are reshaped. Furthermore, a locally connected organization persists over the time precluding to evolve towards a long-distance integrative architecture.

Nevertheless, the PT brain shows resilience and plasticity, driven through an interhemispheric connectivity and alternative WM routes. Both occur in structural and functional networks. In this regard, plasticity is assumed to be positive and reflects a compensatory mechanism. However, the restructuration to an atypic circuitry could also be interpreted as a “maladaptive plasticity”. Indeed, plasticity is a process based on experience that nurtures and shapes the brain. This occurs as early as the neonatal period, and can be beneficial or detrimental. More interestingly, part of the experience is directly derived from neonatal interventions of care providers, which bring new perspectives in the management of PT.

Cerebral MRI constitutes a versatile and efficient means of investigation to exhaustively explore the brain. Effective connectivity studies go beyond the scope of this review as well as gyrification and volumetry. However, those are also influenced by prematurity and plasticity factors [102–104]. Non-invasive neuroimaging techniques, such as magnetoencephalography which measures neural activity may also be coupled with MRI [105]. Finally, deep learning has been introduced in various radiological applications and brings new promises [106]. In other words, the field of research and application of brain MRI is wide. Its evolution follows the strides in computing and data processing. Thus, the perspectives seem endless, and give MRI a key role in neonatal neuroscience. The challenge remains to correlate those invisible alterations to subsequent neurodevelopment, especially the actual rise of cognitive and executive function disorders. As a consequence, methodology in MRI assessment is critical, and further longitudinal studies are needed before drawing any recommendations for the neonatal plan of care.

Author Contributions: P.V.V., L.H.-P. and G.L. conceptualized the idea, P.V.V., M.A., L.H.-P., G.L. wrote the manuscript, P.V.V., M.A., B.M., J.B., N.B., L.H.-P. and G.L. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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OPEN ACCESS

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SPECIALTY SECTION

This article was submitted to
Neonatology,
a section of the journal
Frontiers in Pediatrics

RECEIVED 04 June 2022

ACCEPTED 30 August 2022

PUBLISHED 20 September 2022

CITATION

Vo Van P, Beck J, Meunier H, Venot P,
Mac Caby G, Bednarek N and Loron G
(2022) Assessment of brain
two-dimensional metrics in infants
born preterm at term equivalent age:
Correlation of ultrasound scans with
magnetic resonance imaging.
Front. Pediatr. 10:961556.
doi: 10.3389/fped.2022.961556

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Assessment of brain two-dimensional metrics in infants born preterm at term equivalent age: Correlation of ultrasound scans with magnetic resonance imaging

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Context: Developing brain imaging is a critical subject for infants born preterm. Impaired brain growth is correlated with poor neurological outcomes, regardless of overt brain lesions, such as hemorrhage or leukomalacia. As magnetic resonance imaging (MRI) remains a research tool for assessing regional brain volumes, two-dimensional metrics (2D metrics) provide a reliable estimation of brain structures. In neonatal intensive care, cerebral ultrasound (cUS) is routinely performed to assess brain integrity. This prospective work has compared US and MRI accuracy for the measurement of 2D brain metrics and identification of overt injuries.

Methods: MRI and cUS were performed at term equivalent age (TEA) in infants born before 32 weeks of gestation (GW). Demographical data and results of serial cUS (Neonatal Intensive Care Unit [NICU]-US) performed during hospitalization were gathered from medical charts. Blinded, experienced senior doctors reviewed the scans for both standard analysis and standardized, 2D measurements. The correlation of 2D metrics and inter-/intraobserver agreements were evaluated using Pearson's coefficient, Bland-Altman plots, and intraclass coefficient (ICC), respectively.

Results: In total, 102 infants born preterm were included. The performance of "TEA-cUS and NICU-cUS" when compared to "TEA-MRI and NICU-cUS" was identical for the detection of high-grade hemorrhages and close for low-grade ones. However, TEA-MRI only detected nodular lesions of the white matter (WM). No infant presented a cerebellar infarct on imaging. Intra- and inter-observer agreements were excellent for all 2D metrics except for the corpus callosum width (CCW) and anteroposterior vermis diameter. MRI and cUS showed good to excellent correlation for brain and bones biparietal

diameters, corpus callosum length (CCL), transcerebellar diameters (TCDs), and lateral ventricle diameters. Measures of CCW and vermis dimensions were poorly correlated.

Conclusion and perspective: The cUS is a reliable tool to assess selected 2D measurements in the developing brain. Repetition of these metrics by serial cUS during NICU stay would allow the completion of growth charts for several brain structures. Further studies will assess whether these charts are relevant markers of neurological outcome.

KEYWORDS

brain, cranial ultrasound, MRI, brain imaging, preterm, metrics

Introduction

Brain magnetic resonance imaging (MRI) has brought new insight into the understanding of preterm neuropathology, becoming a gold standard in research and routine healthcare.

Overt lesions, such as white matter (WM) impairment and intraventricular hemorrhages (IVHs), account for a large part of severe, cognitive, and motor sequelae in preterm infants (1, 2). Impaired regional brain growth that has been documented in preterm infants with or without brain injury is also known to be an independent factor of poor neurologic development (3). Segmentation of cerebral structures in three dimensions has remained a research tool until now as it requires specific, additional computational processing. Two-dimensional cerebral metrics, such as bifrontal, biparietal, and transcerebellar diameters (TCDs), are well correlated with regional brain volumes (4) and can be assessed routinely. Besides, interhemispheric distance (IHD) and subarachnoid space are representative of the cerebral spinal fluid total volume. Scoring systems combining both qualitative injuries and cerebral measures have been validated (5–7).

Serial cerebral ultrasound (cUS) screening during hospitalization is recommended by most neonatal scientific societies to depict IVH or extensive WM lesions (8, 9). To our knowledge, neither 3D nor 2D cUS is used as a routine practice to assess the size or growth of brain structures in preterm infants. Some studies have reported the feasibility of US to assess the 2D measurement of brain structures, such as corpus callosum and cerebellar vermis measurements in this population (10, 11). The aim of this prospective study was to (1) compare cUS and MRI accuracy for measurement of 14 2D measurements that included parenchymal and extraparenchymal spaces dimensions, at term equivalent age (TEA) and (2) assess cUS performance for identifying brain lesions when compared to the gold standard MRI.

Materials and methods

Subjects

Infants born before 32 weeks of gestation (GW) were prospectively included between June 2013 and March 2014, upon admission to the level III Neonatal Intensive Care Unit (NICU) (Institut Alix de Champagne, Reims). Exclusion criteria consisted of brain malformations, absence of healthcare coverage, and parental non-agreement. The Local Institutional Review Board approved the study protocol on 6 May 2013.

Clinical data

Clinical data were gathered from medical charts. Antenatal steroids, birth weight, gender, Clinical Risk Index for Babies II (CRIB II) score, any need for inotropic support and ventilatory requirement at 36 weeks post-menstrual age (PMA), and use of post-natal steroids (hydrocortisone or dexamethasone) were collected. Chorioamnionitis diagnosis was based on placental bacterial and anatomopathological study and the presence of increased maternal fever or biological inflammatory syndrome (maternal C-Reactive protein superior or equal to 15 mg/100 ml). Early and late-onset sepsis were defined as a positive bacterial blood culture and a positive biological inflammatory syndrome, before and after 72 h of life, respectively. Necrotizing enterocolitis was classified according to Bell criteria.

Sequential cUS (thereafter: NICU-cUS) was performed according to the protocol of the unit: within the first 3 days of life, at the end of the first week, and then once every 2 weeks by neonatal senior doctor. cUS scans included sagittal and coronal acquisition through the anterior fontanel and mastoid acoustic window. Reports of cUS performed during hospitalization were retrospectively gathered from medical charts.

Imaging acquisition

Cerebral MRI and cUS were performed the same day, at TEA (i.e., superior or equal to 37 PMA). All examinations were anonymized.

Magnetic resonance imaging acquisition

A 3 Tesla Philips Achieva System with 8 channel head coils (Philips, Best, Netherlands) was used for cerebral MRI. The examination was conducted after feeding, using an MRI-compatible incubator, without sedation being administered. Conventional MRI study included sagittal T1-weighted spin echo (3 mm thickness; repetition time (TR), 500 ms; echo time (TE), 10 ms; field of view (FOV), 200 × 200 mm; matrix, 236 × 200), axial T1-weighted spin echo (3 mm thickness; TR, 600 ms; TE, 10 ms; FOV, 190 × 150 mm; matrix, 284 × 160), 3D T1-weighted gradient echo (1 mm thickness; TR, 9 ms; TE 4.6 ms; FOV 220 × 199 mm; matrix, 250 × 220), axial T2-weighted spin echo (3 mm thickness; TR, 3,000 ms; TE, 80 ms; FOV, 180 × 130 mm; matrix, 340 × 195), coronal T2-weighted spin echo (3 mm thickness; TR, 4,600 ms; TE, 150 ms; FOV, 160 × 160 mm; matrix, 204 × 204), and axial T2-weighted gradient echo (3 mm thickness; TR, 919 ms; TE, 16 ms; FOV, 200 × 157 mm; matrix, 224 × 139; angle lever 18).

Magnetic resonance imaging analysis

Two senior medical doctors, a pediatric radiologist (GMM) and a neuro-neonatologist (NB), conducted their own independent reviews of the MRI scans, which were displayed through IMPAX 6.0 DICOM browser (Agfa, Belgium). They were blinded to clinical data and cUS results.

Qualitative magnetic resonance imaging analysis

The qualitative injury scoring system focused on WM, cerebellum, cortex, lesions, and IVH sequelae. WM and cerebellar injury were evaluated for (i) nodular or cystic, (ii) unilateral or bilateral, and (iii) focal or extensive lesions. IVH sequelae were defined according to Kidokoro's classification (3). IVH was classified using Papile classification (12). Grade I and II IVHs were classified as minor IVH.

Brain metrics

In total, 14 variables were assessed (Figures 1, 2) following previously published methods (3, 4). Tissue metrics included bifrontal diameter (BFD), brain and bone biparietal diameter (BPD), TCD, CCL, corpus callosum width (CCW), and anteroposterior diameter and height of vermis (APVD). Fluids metrics comprised pericerebral spaces (left and right subarachnoid spaces, IHD) and intracerebral spaces (left and right lateral ventricles, fourth ventricle diameters).

Ultrasound acquisition

Cerebral US was performed on the same day as the MRI at TEA by PVV. cUS acquisition and analysis were blinded to MRI results and clinical data. Images were recorded using the Sequoia Ultrasound System (Siemens, Erlangen, Germany) with 8 and 12 MHz probes.

Conventional imaging protocol for qualitative analysis included five coronal views in the plane of (1) the orbits, (2) the third ventricle, (3) the fourth ventricle, (4) the bodies of lateral ventricles, and (5) the occipital lobe, and five sagittal-parasagittal views depicting (1) midline, (2) left lateral ventricle, (3) left paraventricular WM, (4) right ventricle, and (5) right paraventricular WM. All those images were acquired through the anterior fontanel.

Acquisition of scans followed the protocol described in Figures 1–3. It comprises four coronal views (Plane A at the level of the orbits just in front of the corpus callosum; Plane B covering the temporal lobes, the parietal lobes, the third ventricle, and the anterior part of the brainstem; Plane C depicting the lateral ventricles and the cerebellum at its maximum width, just behind the fourth ventricle; and Plan D displaying the lateral ventricles posterior) and one midline, sagittal view passing through the anterior fontanel showing the corpus callosum and the vermis. An additional view of the cerebellum through the mastoid was recorded.

Ultrasound imaging analysis

A senior neonatologist (PVV) reviewed the cUS and performed the 14 measurements (Figures 1–3). A second senior neonatologist (NB) independently reviewed a set of 30 examinations for qualitative lesions and quantitative evaluation. Readers were blinded from clinical and MRI data.

Qualitative analysis of cerebral ultrasound

The qualitative analysis assessed the presence of IVH and grade and the presence of nodular or cystic lesions of WM and cerebellum.

Quantitative analysis of cerebral ultrasound

Bifrontal diameter, brain and bone BPD, left and right subarachnoid spaces and lateral ventricles, and IHD were measured from the coronal views (Figures 1, 3). CCL and CCW, fourth ventricle diameter, and APVD were measured from the sagittal views (Figures 2, 3). TCDs were measured from the coronal and mastoidian views (Figures 2, 3) (13).

Statistical analysis

Statistical analysis was performed with Statistical Analysis R Software 3.2.5, Free Software Foundation's GNU General Public License. Sensitivity (Se) and specificity (Sp) criteria were used to evaluate the accuracy of TEA-cUS for assessing

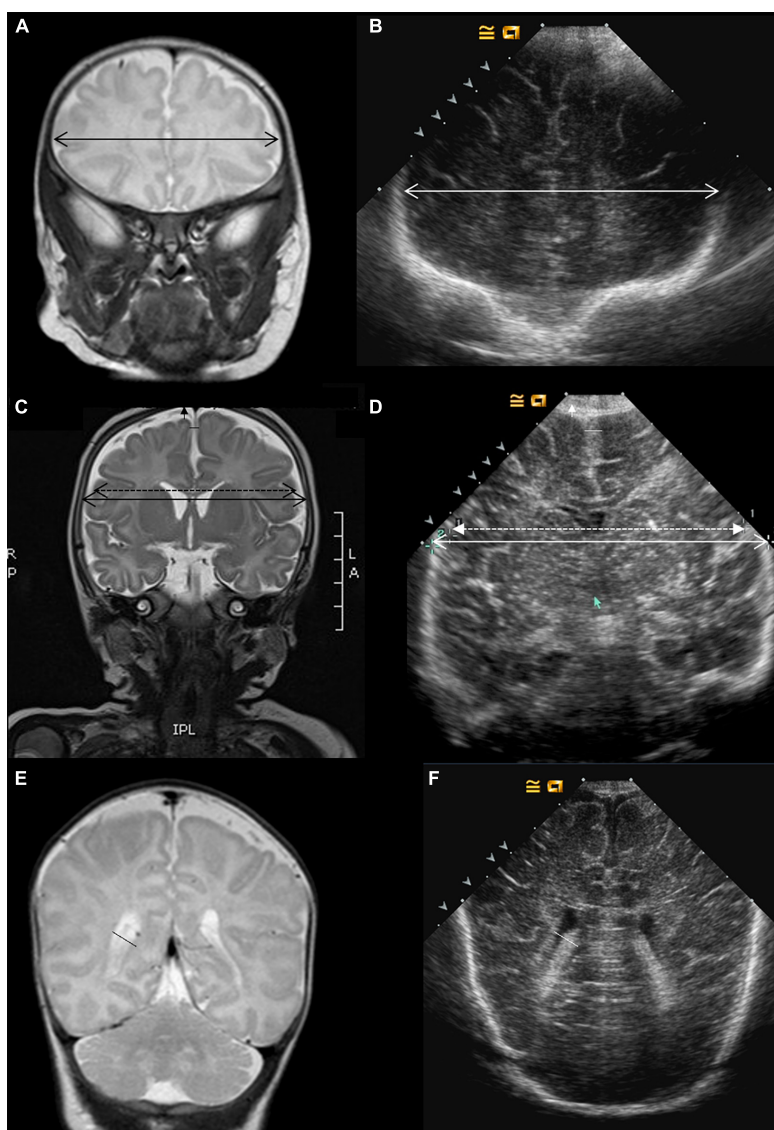


FIGURE 1

Imaging metrics protocol. MRI and ultrasound coronal views. The following metrics were compared on coronal, T2-weighted MRI sequences (A,C,E) and ultrasound scans (B,D,F): bi-frontal diameter (A,B), and lateral ventricle diameter (E,F). On panels (C,D), the bone parietal diameter is shown in plain double head arrow and the brain parietal diameter in the discontinued double head arrow. Measurements of extra-cerebral spaces include the subarachnoid (vertical arrowhead) and the inter-hemispheric spaces (horizontal space).

qualitative lesions by comparison with that of TEA-MRI. The accuracy of metrics measurements by cUS when compared to MRI was evaluated based on Pearson's correlation coefficient and Bland-Altman Plot. Inter- and intraobserver agreements were calculated with intraclass correlation coefficient (ICC) from a sample of 25 MRI and thirty cUS scans. They were analyzed independently by two experienced reviewers, each independently from the other, and clinical data. Analysis of scans for the intraobserver agreement took place 1 month apart. A threshold of 0.7 and above was considered as a good correlation for both Pearson's correlation coefficient and ICC.

Results

Subjects

In total, 102 preterm infants born before 32 GW were included. The clinical characteristics of the population are detailed in **Table 1**. The mean gestational age at birth was 28.9 weeks (± 2.1) with a mean birth weight of 1,127 g (± 313). The mean PMA at cerebral imaging was 39.3 weeks (± 1.7).

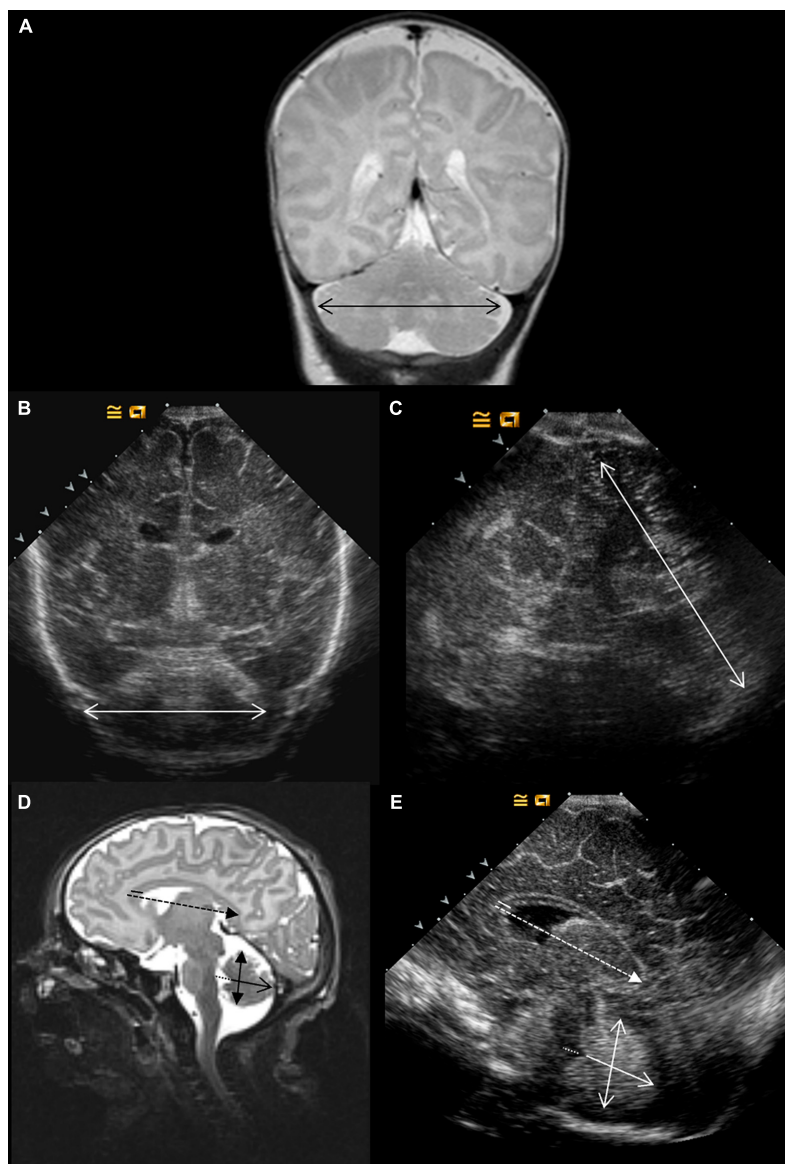


FIGURE 2

Imaging metric protocol. Coronal and sagittal views. On T2-weighted MRI, the transcerebellar diameter is measured on a coronal view (A). Two acoustic windows were compared on ultrasound: coronal (B) and mastoid planes (C). On T2-weighted MRI (D) and ultrasound, sagittal view (E) were assessed the corpus callosum length (discontinued arrow) and width (plain line), the anteroposterior vermian diameter (plain arrow) and height (double head arrow), and the fourth ventricle diameter (discontinued line).

Qualitative analysis of brain imaging

Overall, 55 patients presented one or more qualitative anomalies on imaging (Table 2). Mild IVH (IVH grade I or II) was reported in 27 patients, grade III IVH in four, and IVH plus parenchymal infarction for one (grade IV). Nine patients were diagnosed with nodular WM lesions and three with cystic WM lesions. Nodular cerebellar injuries were found in nine patients. No cerebellar infarction was observed in this population.

The Se and Sp of TEA-cUS reached 11.1 and 100% for nodular WM lesions and 100/100% for cystic WM lesions. Sensitivity and Sp to diagnose mild IVH sequelae (I and II grades), III grade, and IV IVH were 63 and 93.3%; 50 and 100%; and 100 and 100%, respectively. No nodular cerebellar lesions were identified using US scans.

The combination of TEA-MRI and NICU-cUS identified 52 patients with qualitative anomalies, whereas the combination of NICU- and TEA-cUS identified 44 of those patients. When adding data from NICU-cUS to TEA-MRI, ten more mild IVH

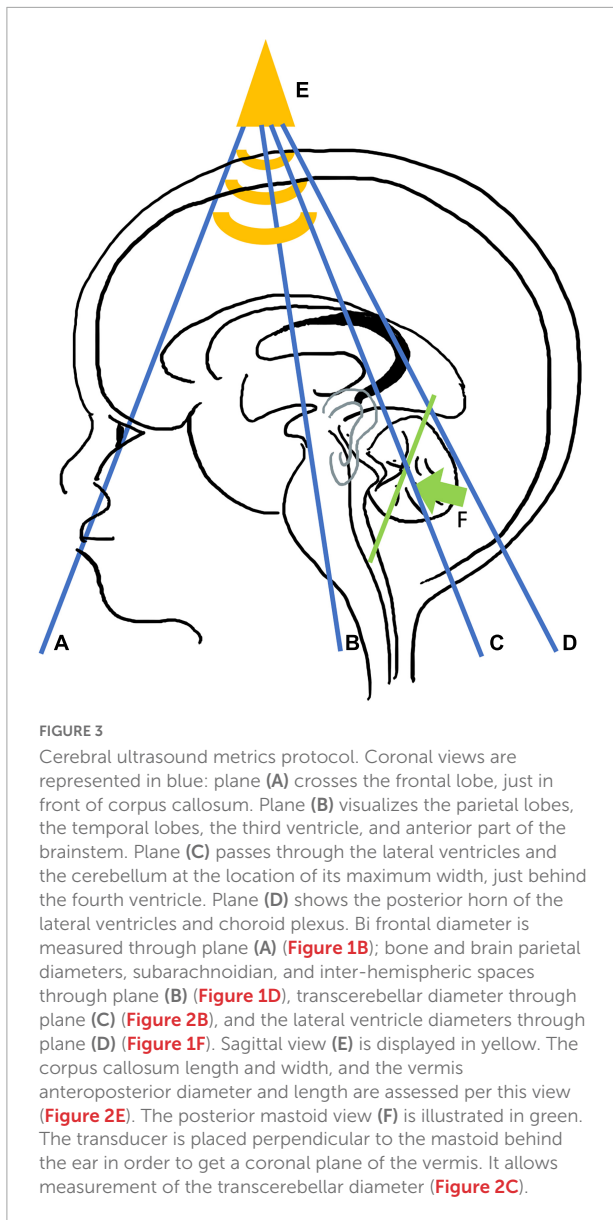


FIGURE 3

Cerebral ultrasound metrics protocol. Coronal views are represented in blue: plane (A) crosses the frontal lobe, just in front of corpus callosum. Plane (B) visualizes the parietal lobes, the temporal lobes, the third ventricle, and anterior part of the brainstem. Plane (C) passes through the lateral ventricles and the cerebellum at the location of its maximum width, just behind the fourth ventricle. Plane (D) shows the posterior horn of the lateral ventricles and choroid plexus. Bi frontal diameter is measured through plane (A) (Figure 1B); bone and brain parietal diameters, subarachnoid, and inter-hemispheric spaces through plane (B) (Figure 1D), transcerebellar diameter through plane (C) (Figure 2B), and the lateral ventricle diameters through plane (D) (Figure 1F). Sagittal view (E) is displayed in yellow. The corpus callosum length and width, and the vermis anteroposterior diameter and length are assessed per this view (Figure 2E). The posterior mastoid view (F) is illustrated in green. The transducer is placed perpendicular to the mastoid behind the ear in order to get a coronal plane of the vermis. It allows measurement of the transcerebellar diameter (Figure 2C).

were identified, as well as two more grade III IVH. When adding data from serial neonatal cUS to TEA-cUS, 11 mild IVH and four grade III IVH were further identified. When NICU-cUS was combined with TEA-cUS, the rate of normal examination was decreased from 76 to 56.9%. Finally, Se values of “TEA-MRI and NICU-cUS” and “TEA-cUS and NICU-cUS” for identifying patients with qualitative anomalies were 94.5 and 80%, respectively.

Brain metrics at term equivalent age

MRI and cUS brain metrics values and their correlation are reported in Table 3. Cerebral MRI and cUS show a reliable correlation (Pearson’s correlation coefficient ≥ 0.70)

TABLE 1 Demographic data of preterm included.

Infant characteristics	<i>n</i> = 102
Gestational Age, weeks	28.9 (2.1)
Birth weight, g	1127 (313)
Sex ratio	1.04
IUGR < 10 th percentile	25 (24.5%)
Antenatal steroids	94 (92.1%)
Chorioamnionitis	15 (14.7%)
CRIB II score	10.5 (3.4)
Postnatal use of steroids	11 (10.8%)
Need for inotropic support	9 (8.8%)
Necrotizing enterocolitis	9 (8.8%)
Early onset sepsis	18 (18.6%)
Late onset sepsis	34 (33.3%)
Need for ventilation at 36 postmenstrual age	21 (20.6%)
Postmenstrual age at MRI, weeks	39.3 (1.7)

IUGR, intrauterine growth restriction; CRIB, Clinical Risk Index for Babies. Data are expressed in mean (SD) or in number (%).

for brain and BPDs, length of corpus callosum, TCD through the coronal window, and diameter of lateral ventricles. The correlation of MRI and cUS measures was only moderate for brain/BPDs ratio, TCD through mastoid view, IHD, and right and left subarachnoid spaces ($0.4 \leq$ Pearson’s correlation coefficient < 0.7). Measures of BFD, CCW, anteroposterior vermis diameter, and fourth ventricle diameter presented low correlation (Pearson’s correlation coefficient < 0.4). Plots of correlation are reported in Figure 4, and Bland-Altman plots of MRI vs. cUS metrics are available in Supplementary Data 1.

Reliability of measurements

Inter- and intraobserver ICCs for MRI and cUS are reported in Table 4.

Intraobserver correlation coefficients for MRI metrics were excellent (above 0.7). Inter-observer ICC was higher than 0.7 for most of the metrics, except for CCW (0.547) and vermis height (0.681).

Intraobserver and inter-observer correlation coefficients for cUS were excellent for all metrics except the CCW (ICC 0.623 and 0.625, respectively). Unlike the MRI results, inter-observer reliability of vermis height was excellent.

Discussion

To our knowledge, this is the first study that assesses the correlation between US and MRI 2D measurements of the brain—or “metrics”—in infants born preterm. Correlation of those metrics is good to excellent for

TABLE 2 Qualitative study of brain imaging.

102 patients	Overall	TEA MRI & NICU cUS	TEA US & NICU cUS	TEA MRI	TEA cUS	NICU cUS
Exams with lesion (s)	55 (54%)	52 (51%)	44 (43.1%)	49 (48%)	29 (28%)	25 (24%)
IVH (or sequelae of)						
Mild (grade 1 and 2)	39 (38.2%)	37 (36.3%)	33 (32.4%)	27 (26.5%)	22 (21.6%)	17 (16.6%)
Grade 3	6 (5.9%)	6 (5.9%)	6 (5.9%)	4 (3.9%)	2 (2%)	5 (4.9%)
Grade 4	1 (1%)	1 (1%)	1 (1%)	1 (1%)	1 (1%)	1 (1%)
Nodular WM lesions	9 (8.8%)	9 (8.8%)	1	9 (8.8%)	1 (1%)	0
Cystic WM lesions	3 (2.9%)	3 (2.9%)	3	3 (2.9%)	3 (2.9%)	2 (2%)
Nodular cerebellar lesions	9 (8.8%)	9 (8.8%)	0	9 (8.8%)	0	0

IVH, intraventricular hemorrhage; WM, white matter; TEA, term-equivalent age; NICU, Neonatal Intensive Care Unit; cUS, cerebral ultrasound. Data are presented as numbers (%).

TABLE 3 Comparison of metrics: MRI vs. cUS.

Total no = 102	Measurements, mm		Correlation Pearson <i>r</i> [CI 95%]
	MRI	Ultrasound	
Bi-hemispheric diameter			
Bifrontal diameter	68.07 (5.5)	69.6 (4.6)	0.45 [0.29–0.59]
Brain biparietal diameter	78.25 (5.4)	79.2 (4.9)	0.81 [0.73–0.87]
Bone biparietal diameter	82.57 (5.8)	82.6 (5.3)	0.87 [0.82–0.91]
Ratio brain/bone	0.95 (0.02)	0.97 (0.02)	0.57 [0.38–0.77]
Corpus callosum			
CC length	41.32 (3.8)	41.4 (3.8)	0.85 [0.80–0.90]
CC width	3.51 (0.8)	2.7 (0.7)	0.30 [0.12–0.47]
Cerebellum			
Transcerebellar diameter (CUS: Coronal window)	54.21 (3.8)	58.8 (3.8)	0.71 [0.60–0.80]
Transcerebellar diameter (CUS: Mastoid window)	54.21 (3.8)	56.7 (3.9)	0.67 [0.54–0.76]
Vermis height	25.04 (2.2)	27.0 (3.2)	0.30 [0.11–0.47]
Ant-post vermis diameter	16.16 (1.8)	18.3 (2.7)	0.36 [0.17–0.52]
Pericerebral spaces			
Inter-hemispheric distance	4.79 (2.4)	4.1 (2.2)	0.69 [0.57–0.78]
R sub-arachnoid space	4.96 (1.9)	3.3 (1.9)	0.60 [0.46–0.71]
L sub-arachnoid space	5.09 (1.9)	3.0 (1.8)	0.62 [0.48–0.73]
R lat ventricle diameter	8.99 (2.4)	7.4 (1.6)	0.79 [0.70–0.85]
L lat ventricle diameter	9.28 (2.3)	7.8 (2.1)	0.73 [0.61–0.80]
Fourth ventricle diameter	5.89 (0.9)	6.9 (1.1)	0.43 [0.25–0.58]

Measurements are expressed in ml presented as mean (SD). CC, corpus callosum; cUS, cerebral ultrasound; R/L right/left, ant-post, anteroposterior; lat, lateral.

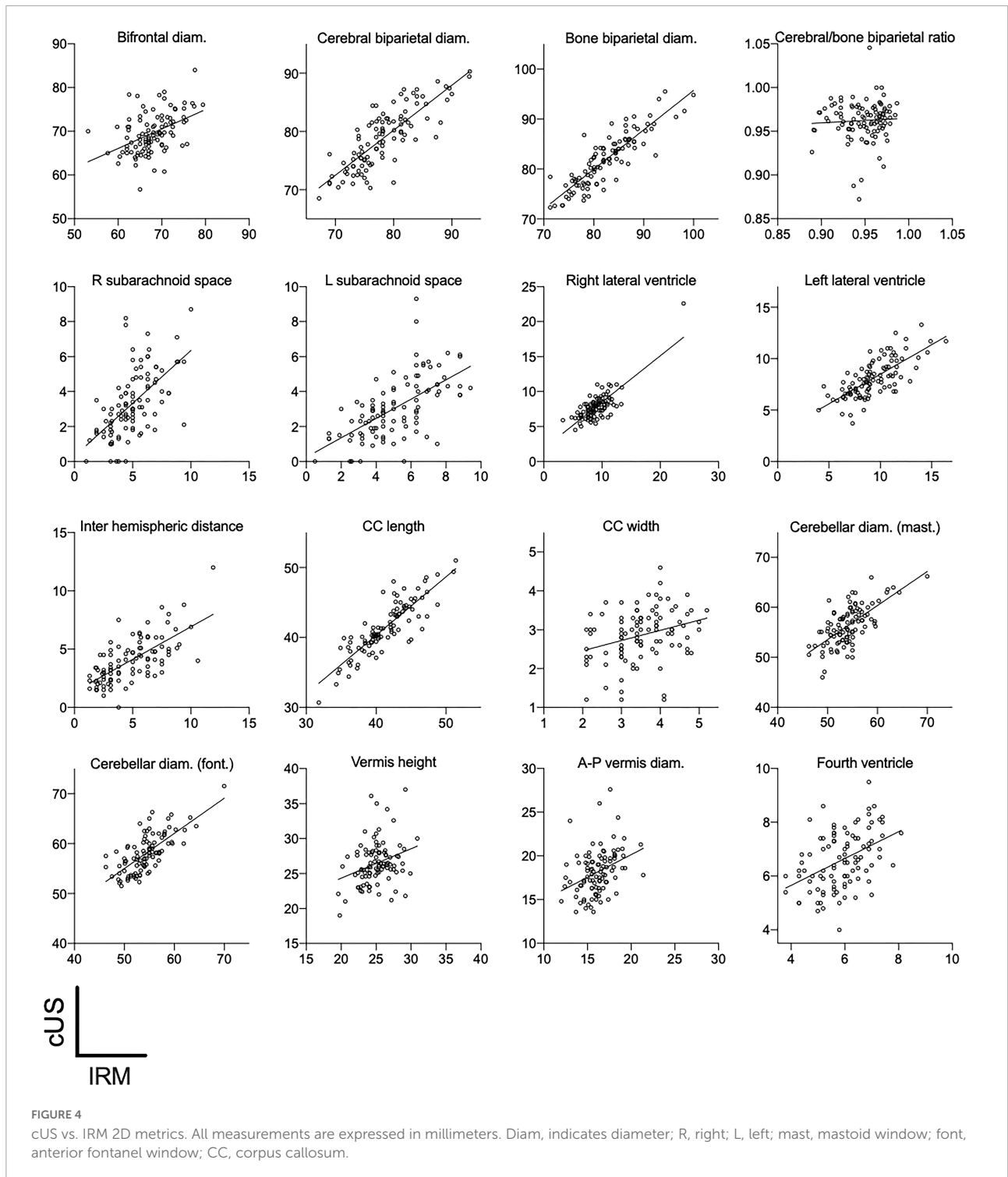
brain and BPDs, CCL, cerebellar diameter, and most of the measures of cerebrospinal fluid (CSF) spaces. Accuracy is lesser for CCW, vermian dimensions, fourth ventricle, and BFDs. Inter-reproducibility and intraobserver reproducibility are excellent except for CCW for both techniques and vermis height for MRI inter-observer reliability only.

The qualitative analysis demonstrates that TEA-MRI depicts more nodular lesions when compared to cUS in cerebral WM and cerebellum, even when combined with results of serial cUS acquired during the NICU stay.

Qualitative study

Moderate to severe WM lesions on MRI predict cerebral palsy with Se between 60 and 77% and Sp between 79 and 96%. They also diagnose a probability of severe neurologic impairments (involving blindness or auditory deficit) with Se and Sp between 53 and 82% and 82 and 85%, respectively (1, 5–7).

In the study's sample, moderate to severe brain WM lesions were present in 12% of patients. Twelve patients showed periventricular leukomalacia (PVL): nine with nodular WM lesions



and three with cystic lesions. Four had grade III IVH and one post-hemorrhagic infarction. These results are relatively consistent with the findings of large cohorts (8).

We did not observe any cerebellar hemorrhage among the included patients, either on US imaging or MRI. In our experience, the mastoidian acoustic window is more efficient for

cerebellar imaging, compared to the transfontanellar acoustic window. This has been corroborated elsewhere (9). Cerebellar infarction, occurring especially in the first weeks of life of babies born extremely preterm, can have a very serious impact on neurological outcomes and should be screened during serial cUS (10).

TABLE 4 MRI and cUS intra- and inter-observer ICCs.

	MRI ICC		cUS ICC	
	Intra-observer	Inter-observer	Intra-observer	Inter-observer
Bi-hemispheric diameters				
Bifrontal diameter	0.968	0.780	0.966	0.969
Cerebral biparietal diameter	0.964	0.864	0.963	0.980
Bone biparietal diameter	0.979	0.962	0.963	0.952
Corpus Callosum				
Length	0.983	0.898	0.964	0.957
Width	0.737	0.547	0.623	0.625
Cerebellum				
Transcerebellar diameter (coronal window for cUS)	0.963	0.968	0.987	0.986
Transcerebellar diameter (mastoid window for cUS only)	0.963	0.968	0.832	0.860
Vermis height	0.862	0.681	0.902	0.902
Anteroposterior vermis diameter	0.871	0.772	0.719	0.713
Pericerebral spaces				
Fourth ventricle diameter	0.898	0.855	0.808	0.810
Interhemispheric distance	0.977	0.800	0.983	0.983
Right sub arachnoid space	0.984	0.710	0.913	0.931
Left sub arachnoid space	0.974	0.700	0.958	0.965
Right ventricular diameter	0.931	0.870	0.953	0.868
Left ventricular diameter	0.902	0.884	0.986	0.835

ICC, intraclass coefficient; cUS, cerebral ultrasound.

Cerebral US may find 79% of major abnormalities related to cerebral palsy (i.e., cystic PVL and major IVH) with sensitivity and specificity of 76 and 95%, respectively (6, 11). Compared to susceptibility-weighted imaging MRI sequence, Se and Sp of cUS were 100 and 93% to detect grade III IVH and 66.7/100% for grade IV IVH (12). In this work, T1 and T2 sequences were found to be as efficient as susceptibility-weighted imaging sequences. The Se and Sp of cUS at TEA to diagnose grade III and IV IVHs were, respectively, 50/100 and 100/100%. Retrospectively, some images interpreted as grade III IVH on MRI were classified as a ventricular enlargement on cUS, as no sequelae of bleeding were identified.

Ultrasound had failed to identify nodular PVL; only one out of nine led to a low Se of 11%. Sensitivity and Sp of Us as compared to MRI for the diagnosis of nodular PVL were known to be low: from 26 to 38% and 85 to 96%, respectively (13, 14). This may be a limitation of technique and resolution itself.

When combining data from a sequential US performed during hospitalization with the term-equivalent US, the accuracy of an “all-ultrasound protocol” to detect IVH from grades I to III is improved. However, performance remains low for nodular lesion detection. These results must be interpreted with caution due to the retrospective collection of NICU-cUS.

Overall, TEA-brain MRI proves to be a superior means of diagnosis than TEA-cUS for a complete detection,

quantification, and characterization of qualitative WM abnormalities, especially nodular lesions.

Metrics as a surrogate of qualitative analysis

Evaluating brain growth in the preterm population is of critical interest. Premature birth disrupts brain growth independently of the development of WM lesions or IVH (15, 16). Many 3D segmentation brain MRI studies demonstrated decreased cerebral volume across many structures in infants born preterm (17, 18). These alterations persist after childhood and are firmly correlated with cognitive issues and behavioral disorders (15, 19–22). However, 3D segmentation remains unavailable in routine practice while 2D metrics provide a reliable and convenient way to obtain markers of brain growth. A strong correlation was found between BFD, BPD, and TCD and total brain and cortical gray matter and volumes (4, 23). IHD was also correlated with total CSF volume (24). Finally, enlarged lateral ventricle is a common feature of WM loss of substance (13, 14).

This study shows that cUS provides good to excellent estimation of cerebral and fluid metrics by comparison to MRI: bifrontal, biparietal, TCDs, CCL, ventricle diameters, and the IHD.

Corpus callosum is a large WM bundle involved in the inter-hemispheric communication. Premature birth impacts callosal growth and disturbs WM connectivity and the myelination process (20). Such alteration of callosal growth and microstructural disorganization has been correlated with a worse cognitive and motor outcome than a control population (20, 22, 25). In a cohort of very preterm infants <30 weeks, the majority of survivors did not have severe brain injuries but an altered biparietal diameter and IHD (24). Both altered metrics were associated with adverse neurologic outcomes at 2 years old (23, 24). An enlargement of the IHD may reflect an early-impaired cortical growth with a preserved skull growth (preserved head circumference). The consequences of the impaired cortical growth on skull growth manifest themselves at a later stage in life, therefore, head circumference at term equivalent age was not correlated with adverse outcome (26).

In the absence of CSF circulation impairment, increased lateral ventricle diameter in preterm infants is a marker of atrophy and WM loss. Ventricle enlargement is known to be correlated with pejorative cognitive and motor outcomes at 2 years old (27). Moreover, the relation with cognitive scores remained statistically strong after the exclusion of concomitant brain pathology (cystic PVL, IVH, deep gray matter, or cerebellum lesions) with a trend of alteration in a fine motor score (27).

The cerebellum is an area of particular interest in preterm infants because of its acute growth during the third trimester of the pregnancy. Cerebellar diameter at term equivalent age seems to be independently related to cognitive and motor outcomes (25, 27). This finding was also supported by the correlation between cerebellar abnormal dimensions and motor function at 7 years old (10). The cerebellar is thought to support cerebral hemisphere development with a precise topographic correlation; a cerebellar lesion may disrupt the development of cortical area. This phenomenon has been reported as a “cerebellar diaschisis” (10, 19). Cerebellar metrics were shown to be interrelated with deep GM areas whose basal ganglia and thalami receive afferences from the cerebellum (27).

In total, 14 metrics have been evaluated on MRI and cUS. The corpus callosum, parietal lobes, inter-hemispheric space, and transversal diameter of the cerebellum were the structures most accessible by US probes through the anterior or the mastoid window, which explains their high ICC scores. An anterior fontanel window was most appropriate to view supratentorial structures. The third ventricle is a straightforward landmark to spot by US. On MRI scans, it is lined up with the cochleas, which is the reference landmark described by Tich et al. to measure the biparietal diameter, IHD, and the subarachnoid spaces (4). There was greater variation in the evaluation of subarachnoid spaces although cUS allows to readily view them. One hypothesis is that the pressure of the probe on the anterior fontanel might vary the space between

the subarachnoid layer and the cerebral gyrus. The acquisition of the pictures through the frontal lobe differs between MRI and US. MRI slices are acquired perpendicularly to the cerebral axis whereas the US scans through the anterior fontanel constitute a vector-based sector, which may be the cause of the discrepancy between the US and MRI measurements. Regarding the CCW, the correlation score is low, probably due to the under-5 ml size of the structure.

For some infra-tentorial metrics, US failed to get a reliable correlation with MRI. The vermis and the fourth ventricle were not outlined well enough to allow accurate measurement. The mastoid window provides better resolution and is considered the most accurate US method for the imaging of cerebellar structure as compared to anterior fontanel window (28). The study also corroborates the primacy of the mastoid window in this particular aspect, since it provides a higher ICC TCD score when compared to the standard coronal view. However, the plane in which the measurements are performed differs from that in MRI, which may explain the less compelling correlation of TCD.

In a recent work, Skiöld et al. (29) investigated the validity and prognostic value of a standardized scoring system for TEA-cUS. They compared it to a previously validated scoring system for TEA-MRI (3) and demonstrated comparable performance of TEA-cUS and TEA-MRI for the prognosis of cerebral palsy, and close for the prognosis of severe cognitive delay. The scoring system included qualitative, visual analysis, and “metrics” consisting of measurements of intra- and extra-parenchymal CSF and corpus callosum thickness. One could envision integrating the most reliable US parenchymal metrics described in the present study into such a scoring system and endeavor to further refine the performance of US imaging in establishing neurodevelopmental prognosis, especially regarding the cognitive outcome.

Conclusion

Brain MRI constitutes the gold standard means at term equivalent age for assessing cerebral complications of prematurity. Routine score MRI includes a qualitative assessment of cerebellum, lateral ventricles, cortical folds, deep gray and WM, and quantitative measures of brain structures (24). It has been validated in different preterm populations at term equivalent age and preterm PMA (24, 30). On the other hand, cUS constitutes a useful tool in routine practice as it provides a rapid and handy means of investigation and is mastered by a vast majority of neonatologists. As the assessment of cerebral growth becomes a crucial variable to predict the neurologic outcome, this study demonstrates the feasibility of cUS to evaluate through 2D metrics impaired brain growth. Moreover, US examination can be conducted repeatedly during the hospital stay, allowing for serial assessment of brain growth.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Reims Institutional Review Board. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

PVV, HM, PV, GM, NB, JB, and GL: conceptualization. PVV, HM, PV, GM, and NB: methodology. PVV, NB, JB, and GL: validation. PVV and GL: formal analysis and writing—original draft preparation. GL and NB: writing—review and editing. NB: supervision and project administration. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

We are grateful to the American Committee members for their support of the American Memorial Hospital for over 100

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years. We thank Olivier Vo Van for his careful editing of the manuscript's English version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fped.2022.961556/full#supplementary-material>

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Protocol for a prospective multicenter longitudinal randomized controlled trial (CALIN) of sensory-tonic stimulation to foster parent child interactions and social cognition in very premature infants

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Submitted to Journal:
Frontiers in Pediatrics

Specialty Section:
Neonatology

Article type:
Methods Article

Manuscript ID:
913396

Received on:
05 Apr 2022

Revised on:
16 Jul 2022

Journal website link:
www.frontiersin.org

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

G. Loron, A. Novo and C. Guittard conceived the study and coordinated its design. C. Guittard drafted the manuscript. S. Caillies supervised the writing of the manuscript. All authors approved the final manuscript prior to submission. C. Barbe and C. Gower helped with implementation of the study.

G. Loron is the principal investigator of the protocol. The lead investigators are C. Guittard, G. Loron and A. Novo.

Keywords

neurodevelopment, preterm, Brain, Proprioception, interactions, Clinical Trial, brain imaging, Parenting (MeSH)

Abstract

Word count: 282

Introduction: Premature birth is associated with long-term somatic and neurological disorders, including cognitive, social and behavioral impairments. Moreover, the mothers of infants born preterm exhibit a higher prevalence of anxiety and depressive symptoms after birth. Early rehabilitation, developmental care, and parenting support have already been shown to have a positive impact on neurological outcome. However, no randomized controlled study has so far examined the effects on parenting and long-term neurological outcomes of using proprioceptive stimulation to trigger positive brain plasticity in very preterm babies. The CALIN project will therefore investigate the impact of sensory-tonic stimulation (STS) of extremely preterm infants by their parents on child parent interactions, infants' morphological and functional brain development and subsequent cognition (including social cognition), and parents' anxiety and depressive symptoms in the postpartum period.

Methods and analysis: Infants born between 25 and 32 weeks of gestation will be randomly assigned to the "STS + Kangaroo care" or "Kangaroo care" group. The primary endpoint, Child parent interactions, will be rated at 12 months corrected age using the Coding Interactive Behavior system. Secondary endpoints include: 1/ functional and anatomical brain maturation sequentially assessed during neonatal hospitalization using electroencephalogram (EEG), amplitude-integrated EEG (aEEG), cranial ultrasound and MRI performed at term-corrected age, 2/ social and cognitive outcomes assessed at 15 months, 2, 4 and 6 years, and 3/ parents' anxiety and depressive symptoms assessed at 7 ± 1 weeks after birth, using dedicated questionnaires.

Ethics and dissemination: This study was approved by the French Ethics Committee for the Protection of Persons on 18 October 2021. It is registered with the French National Agency for the Safety of Medicines and Health Products (ANSM; no. 2020-A00382-37). The registry number on ClinicalTrials.gov is NCT04380051.

Contribution to the field

Our article provides a brief overview of the difficulties caused by the context of prematurity in children and their parents. We present the protocol of an early sensory-tonic intervention aimed at limiting these difficulties while overcoming the limits of the interventions already implemented in neonatal intensive care units. We hope that this study will be pertinent to any clinician taking care of premature neonates. We therefore believe that our study will be of interest to a wide audience within the neonatology, neuropediatric, child psychiatric and psychological community. Premature birth is often associated with long-term somatic and neurologic disorders and cognitive and social difficulties with behavioral problems. Moreover, mothers of infants born preterm exhibit a higher prevalence of anxiety-depression symptoms after birth. The CALIN project aims at investigating the effect of an early active sensory-tonic stimulation (STS) - performed by a parent on his/her infant born extremely preterm - on several dimensions : child-parent's interactions, morphological and functional brain development of the child, and his/her further social and cognitive developments and additionally the both parents' anxiety-depression symptoms in postnatal period. If this study validates the positive impact of this early intervention, it could be easily generalized and reproducible, including in so-called low-income countries.

Funding statement

This protocol has been selected and funded by the French PHRC Program.

Ethics statements

Studies involving animal subjects

Generated Statement: No animal studies are presented in this manuscript.

Studies involving human subjects

Generated Statement: The studies involving human participants were reviewed and approved by French Ethics Committee for the Protection of Persons. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Inclusion of identifiable human data

Generated Statement: No potentially identifiable human images or data is presented in this study.

In review

Data availability statement

Generated Statement: Publicly available datasets were analyzed in this study. This data can be found here: [ClinicalTrials.gov NCT04380051](https://ClinicalTrials.gov/NCT04380051).

In review

1 **Protocol for a prospective multicenter longitudinal randomized**
2 **controlled trial (CALIN) of sensory-tonic stimulation to foster parent**
3 **child interactions and social cognition in very premature infants**

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18 **Parenting**

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42

43 **1 Introduction**

44 **1.1 Background**

45 In 2018, the World Health Organization (WHO) estimated that 15 million children are born prematurely
46 each year worldwide. The number of newborns surviving a premature birth has gradually increased
47 over the years (1,2). However, many of those will exhibit varying degree of neurological impairment.
48 In the recent decades, the clinical and morphological picture of brain insults due to prematurity has
49 changed. Classically, extensive clastic injuries (e.g., cystic necrosis of white matter, large infarction of
50 brain parenchyma) most often led to cerebral palsy and/or moderate to severe cognitive impairment.
51 Since the 1990s, however, their incidence has dwindled, and they have been supplanted by less
52 prominent and more diffuse brain damage leading to the loss of vulnerable cells and impaired brain
53 development(3–5).

54 Nowadays, the spectrum of neurodevelopmental outcomes of preterm babies broadly includes deficits
55 in language, gross and fine motor skills, behavior, and cognition (especially executive functions and
56 social cognition). The severity of the clinical picture is related to the degree of prematurity (6–12).
57 Many brain areas undergo a sensitive period of development during the third trimester of gestation and
58 after birth. This is the case of the median prefrontal cortex, temporoparietal junction and posterior
59 temporal sulcus (involved in cognition and social cognition) (13–15), the cerebellum, as well as visual,
60 olfactory and somatosensory pathways (16). Premature birth dramatically modifies the context of this
61 developmental window. During hospitalization, the developing brains of infants born preterm are
62 exposed to stimuli that may be detrimental to their maturation (17) with either too much or too little
63 sensorial input (18). This is referred to as *dystimulation*.

64 Furthermore, premature birth disturbs early infant-parent interactions, and ultimately relationships with
65 others. Preterms are not only separated from their parents, but placed in a stressful, technical, and
66 potentially painful environment. Comorbidities and sedation have a negative physiological impact and
67 reduce their availability for interaction. On the parental side, the idealized postnatal period is replaced
68 by an anxious—and even traumatic—experience (19–21). Parents often express guilt and anxiety about
69 the survival of their child and their parenting skills. A higher prevalence of parental anxiety, postnatal
70 depression and posttraumatic stress disorder has been observed in the mothers of infants born preterm,
71 (22) even up to 18 months after birth (23).

72 Finally, prematurity severely disturbs the ability of both parents and newborns to interact and find
73 reassurance. More than 35% of children born preterm subsequently exhibit insecure attachment
74 behavior in relationships with others (24). Given that studies have demonstrated a longitudinal link
75 between attachment security and cognitive development (25,26), specially social cognition (27,28), it

76 seems important to study the effects of disturbed early interactions on child development, and to test
77 the potential of intervention programs to efficiently minimize these effects.

78 **1.2 Objectives and hypotheses**

79 The overall aim of this study is to assess effect of a sensori-tonic stimulation (STS) provided by parents,
80 associated with kangaroo care, versus kangaroo care alone, on the quality of parent-infant interactions,
81 parental wellbeing, and ultimately brain maturation and cognitive outcome of infants born preterm.

82 **1.2.1 Primary objective**

83 This study will investigate the benefits of early STS, provided by one of the parents on its infant born
84 preterm on the developing interactions between them. We expect to observe stronger interactions in
85 the dyads who practiced STS + kangaroo care, than in the control group (kangaroo care only).

86 **1.2.2 Secondary objectives**

87 The present work will assess, in children born very preterm, the effect of STS+kangaroo care vs
88 kangaroo care alone on: 1/ the morphological and functional brain maturation, 2/ the precursors of
89 cognitive development at 15 months, 3/ psychomotor development at 2 and 4 years, and 4/ cognition
90 including social cognition assessed at 6 years of age.
91 Moreover, this study will assess the impact of the intervention on symptoms of anxiety and depression
92 experienced by the parents of children born preterm.

93

94 **2 METHODS**

95 **2.1 Design**

96 The CALIN study is a prospective, multicentric randomized study. The experimenters who carry out
97 the assessments will be blind to the result of the randomization. Participants will be recruited in French
98 tertiary neonatal centers equipped with Neonatal Intensive Care Units.

99 **2.2 Participants**

100 **2.2.1 Study population**

101 Children born between very preterm and their parents will be eligible if they meet the following
102 inclusion criteria: 1/ the child is inborn, 2/ birth between 25 and 32 weeks of gestation and weight over
103 600g at birth, 3/ the child's hospitalization at the recruiting center is planned until 36 weeks corrected
104 age, 4/ the child's parents have parental authority and have agreed to participate in the study by signing
105 the informed consent, 5/ the child's parents are available physically and mentally to participate in the
106 study and 6/ the child and his/her parents are affiliated to the social security system.

107 The exclusion criteria are the following: 1/ the child is hemodynamically unstable, 2/ the child has a
108 suspected comorbidity (e.g., genetic syndrome, congenital malformation, brain injury, skin pathology),
109 3/ the child was born from a multiple pregnancy, 4/ the child was born anonymously, 5/ the child is to
110 be separated from his/her parents (e.g., assigned to infancy protection services).

111 2.2.2 Sample size calculation

112 The sample size has been calculated so that a two-tailed test will detect a significant difference in the
113 primary endpoint (Coding infant behaviour scale at 12 months corrected age, see below), with a power
114 of 90% and a significance level of 0.05 (alpha value). Forty-eight infants would be required in each
115 group (NQuery 4.0® software). In anticipation of loss to follow-up, the target size has been increased
116 by 20%, such that 60 children will be included in each group, resulting in inclusion of a total of 120
117 infants.

118 2.2.3 Randomization

119 The newborns and their parents will be randomly assigned to either the experimental group or the
120 control group. Those in the experimental group will participate in the STS intervention, in addition to
121 kangaroo care. The control group will benefit from kangaroo care alone. Besides that, the medical and
122 nursing care will be the same, regardless of the outcome of the randomization.

123 2.3 Intervention

124 Sensory-tonic stimulation (STS) refers to a protocolized, tactile, vestibular and kinesthetic stimulation,
125 provided by a parent to its baby born preterm. This proprioceptive stimulation is carried out using an
126 enveloping and continuous touch with a moderate pressure, as described in the literature (29,30). Its
127 characteristics and duration are adapted to the sensorineural immaturity of very premature newborns.
128 Parents will be coached by a trained, designated professional, initially using a mannequin. They will
129 also be shown a training video and given an illustrated training booklet covering all the steps of STS.

130 STS will begin from the 10th day after birth and when the parents feel ready to perform it and will
131 continue until discharge from hospital or 36 weeks corrected age. The preterm infant is either installed
132 in a microbead baby nest in the incubator or on the changing table, depending on the newborn's usual
133 arrangement and maturity. STS should be carried out 5 times per week, 5-15 minutes each time. STS
134 will be performed when the newborn exhibits alertness (State 3 or 4 according to Brazelton (31)). The
135 intervention will be interrupted if signs of discomfort are detected in the newborn or his/her parents.

136 STS will be adapted to the newborn's term and level of development, stratified into three levels (25-30,
137 31-34 and 35-36 corrected age, respectively). During each intervention, a nurse will check the
138 newborn's somatic stability and alertness by filling out a dedicated questionnaire (32). Once a week, a
139 trained psychologist or psychomotor therapist will supervise the parent's gestures and discuss his/her
140 experience.

141 Kangaroo care will be practiced without any limitation in both groups, following local policies. In the
142 experimental group, newborns and their parents will participate in STS in addition kangaroo care.

143 Preterm babies will benefit from the usual neonatal intensive care, following the local policies of each
144 center.

145

146 2.4 Assessments and outcomes

147 An overview of the different assessments and outcomes is provided in **Figure 1**.

148 **2.4.1 Primary outcome**

149 The quality of interactions between the very premature infants and their parents will be scored at 12
150 months corrected age using the Coding Infant Behavior system (CIB) (33). The latter assesses the
151 quality of child-parent interactions by observing the occurrence of behaviors along six dimensions:
152 parental sensitivity, parental intrusion, child's social engagement, child's negative emotionality and
153 engagement, dyadic reciprocity, and negative dyad states. To rate the occurrence of these behaviors,
154 we will record two 15-minute sequences of child-parent interactions during free play. Observations
155 will be double coded by professionals trained to use the CIB, blinded to the randomization group. The
156 main judgment criteria will be the score in "child's social engagement" dimension of the CIB system.
157 The Secondary judgment criteria will be the scores in the other dimensions of the CIB system.

158 **2.4.2 Secondary outcomes**

159 **2.4.2.1 Morphological and functional development of the brain**

160 All the data yielded by the following paraclinical examinations will be anonymized and sent to the
161 main investigating center for centralized review. They will be interpreted by two experts for each
162 technique used, blinded to the randomization group.

163 Serial cerebral ultrasound scans will be performed during the infants' NICU stay, following a
164 standardized protocol. Lesions will be classified: 1/ according to the Papille classification for
165 intraventricular hemorrhages, 2/ according to the De Vries classification for periventricular
166 leukomalacia (34). Moreover, several two-dimensional measurements will be performed on ultrasound
167 scan to assess brain growth: interhemispheric distance, ventricular width, thickness of motor and
168 somatosensory cortex, diameter of thalami and cerebellum.

169 Brain magnetic resonance imaging (MRI) will be performed at term-corrected age including a diffusion
170 sequence, following a standardized protocol of acquisition. Imaging will be interpreted according to
171 Kidokoro (35). Moreover, regional brain volumes will be quantified using semi-automatic, post-hoc
172 segmentation.

173 EEG and amplitude-integrated EEG will be sequentially recorded. Timing of the EEG recordings and
174 their interpretation will follow the guidelines of the French society of electrophysiology (36).

175 **2.4.2.2 Sequential assessment of cognition and psychomotor development**

176 Precursors to cognitive development will be assessed at 15 months corrected age using the "Batterie
177 d'Évaluation Cognitive et Socio-émotionnelle" (37). This psychometric tool assesses cognitive and
178 socio-emotional development in young children up to 24 months, based on standardized activities and
179 standardized sequences of interactions between the psychologist and the child.

180 Child development at 2 and 4 years will be measured using the Ages and Stages Questionnaire (ASQ24
181 and ASQ 48) (38). Those questionnaires, filled by parents, include 30 items assessing five areas of
182 development: communication, gross motor skills, fine motor skills, problem solving, and individual or
183 social skills. A French, validated translation is available, featuring age-appropriate questions
184 corresponding to the child's expected level of development and normative data.

185 Cognitive and social development will be assessed at 6 years using an extensive set of
186 neuropsychological tests and theory of mind tasks: 1/ Global intellectual abilities will be assessed with
187 the Wechsler Intelligence Scale for Children (WISC-V). This scale has five main indices: Verbal
188 Comprehension, Visual Spatial, Fluid Reasoning, Working Memory, and Processing Speed and allows

189 calculation of a global intellectual quotient. 2/ To assess attention, inhibition and mental flexibility,
 190 The NEPSY's Auditory Attention and Associated Responses subtest will performed (39). 3/ The
 191 NEPSY's Sentence Repetition subtest will be used to assesses phonological working memory abilities
 192 through a task where participants have to repeat sentences of increasing length and complexity (39). 4/
 193 The Test of Everyday Attention for Children (TEA-CH)'s Opposite Worlds subtest will attention and
 194 inhibition abilities through a pointing task in response to a verbal cue (40). 5/ The Raven Matrix Test
 195 assesses nonverbal intellectual efficiency through a reasoning by analogy task (41). 6/ Social cognition
 196 will be assessed using Cognitive and Affective theory of mind abilities tasks (42–48).

197 **2.4.2.3 Parental anxiety and depressive symptoms**

198 Anxiety and depressive symptoms will be assessed in both parents at 7 weeks \pm 1 after birth. Maternal
 199 anxiety and depressive symptoms will be measured using the State Trait Anxiety Inventory (STAI-Y),
 200 Parental Stress Index (PSI), Beck Depression Inventory (BDI), Edinburgh Postnatal Depression Scale,
 201 and Modified Perinatal Post Traumatic Stress Disorder Questionnaire. As some of these scales are not
 202 suitable for rating fathers, they will be replaced with non-gender specific scales. Paternal anxiety and
 203 depressive symptoms will therefore be measured using the STAI-Y, PSI, BDI, and Posttraumatic Stress
 204 Disorder Checklist for DSM-5 (49–54).

205 **2.5 Data analysis**

206 First, we will carry out a descriptive analysis of the data, with means and standard deviations, and
 207 counts and percentage for for the quantitative and qualitative variables, respectively. For all
 208 subsequent analysis, the group (experimental vs. control) will be the independent variable. The scores
 209 obtained on each seal will be the dependant variable.

210 **2.5.1 Statistical Analysis of Primary Outcome**

211 The main judgment criteria (score in the "child's social engagement" dimension of the CIB at 12
 212 months) will be compared between the experimental group and the control group using a Student or
 213 Mann-Whitney test, depending on the application conditions.

214 Secondary judgment criteria (scores in the other dimensions of the CIB at 12 months) will be compared
 215 between the experimental group and the control group using a Student or Mann-Whitney test,
 216 depending on the application conditions.

217 **2.5.2 Statistical Analysis of Secondary Outcomes**

218 All secondary outcomes are quantitative measures. For all these measures, we will compare the scores
 219 of the experimental group with those of the control group, according to the distribution of the data,
 220 with parametric or nonparametric tests (Student's t , Mann-Whitney, χ^2 or Fisher's exact tests),
 221 depending on the application conditions. Correlations will be calculated between quantitative
 222 measures.

223 A p value of < 0.05 will be considered statistically significant.

224 Statistical analysis will be performed with easieR software (55).

225

226 **2.6 ETHICAL CONSIDERATIONS**

227 The present study was approved by the French Ethics Committee for the Protection of Persons on 18
228 October 2021. It is registered with the French National Agency for the Safety of Medicines and Health
229 Products (ANSM; no. 2020-A00382-37). The registry number on ClinicalTrials.gov is NCT04380051.

230

231 **3 DISCUSSION**

232 Although prematurity has been identified as a *public health problem* by the WHO since 2010, studies
233 assessing the efficiency of early intervention programs are still sparse.

234 Developmental care and the kangaroo method (also called *skin-to-skin*) have been implemented in
235 many neonatal intensive care units (NICUs). These programs aim to reduce dystimulation, sleep-wake
236 cycle interruptions and enhance affective contact with parents. They have been shown to have many
237 positive effects on weight gain, somatic development, electroencephalographic (EEG) activity (56),
238 medical complications, and duration of hospitalization (57–59).

239 Several teams are now investigating whether the focused enrichment of infants' sensory environment
240 can improve sensory input and, ultimately, brain development. Massage and exposure to sounds such
241 as maternal heartbeat, voices and music are promising avenues that are currently being explored (60–
242 64).

243 The present study will focus on sensory-tonic stimulation (STS). Complementing the standard care
244 designed to protect premature newborns from all the usual dystimulation, the purpose of this STS
245 intervention will be to improve proprioceptive input, which is generally lacking in NICUs. The
246 sensorimotor cortex exhibits activity-dependent plasticity during this crucial period of
247 development(65,66). In the past, tactile stimulation of very premature infants in the form of *massage*
248 has had a positive impacts on physical growth (e.g. weight, height) (29,67), electroencephalographic
249 (EEG) activity maturation [35,37], sleep quality and stress behaviors (e.g. crying, motor
250 agitation)(29,61), physiological measurements (e.g. IGF binding protein-3, glucose, insulin, cortisol
251 and thyroid hormones) (67), and early psychomotor development (69). Interestingly, this kind of
252 intervention may also have a positive impact on parents' moods and skills. Tactile, tender and
253 affectionate contact increases plasma levels of oxytocin and endorphins (associated with feelings of
254 wellbeing and happiness) in both the infants and their parents (61). However, these studies had widely
255 diverging protocols, sample sizes, participants (parents vs. caregivers), and stimulation durations.

256 We are aware that the CALIN protocol has potential limitations and biases. We may be confronted
257 with a potential variability in stimulation time if parents continue to practice STS after the infant has
258 left hospital. We also expect to a risk of loss to follow-up due to the longitudinal design of this study.

259

260 **4 Figures**

261 **Figure 1.** Design of the study, overview of assessments and outcomes.

262

263 **5 Conflict of Interest**

264 The authors declare that the research was conducted in the absence of any commercial or financial
265 relationships that could be construed as a potential conflict of interest.

266 **6 Author Contributions**

267 G. Loron, A. Novo and C. Guittard conceived the study and coordinated its design. C. Guittard
268 drafted the manuscript. S. Caillies supervised the writing of the manuscript. All authors approved the
269 final manuscript prior to submission. C. Barbe and C. Gower helped with implementation of the
270 study.

271 G. Loron is the principal investigator of the protocol. The lead investigators are C. Guittard, G. Loron
272 and A. Novo.

273 **7 Funding**

274 The present research was funded by the French Interregional Clinical Research Hospital Program
275 (PHRC-I).

276 C. Guittard's PhD research is funded by the American Committee of the American Memorial
277 Hospital of Reims.

278 **8 Acknowledgments**

279 We thank the American Committee for supporting the activities of the American Memorial Hospital
280 in Reims for over 100 years.

281 We thank the families who will participate in this study.

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Figure 1.TIFF

Figure 1. Design of the study, overview of assessments and outcomes.
Abbreviations: STS is for sensori-tonic stimulation; WG is for weeks of gestation; *ca* is for corrected age

