

**Réunion annuelle
Axe Rétine**

10 juillet 2007

**Salle Albert Royer
Hôpital Ste-Justine**

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Salle Albert Royer – Hôpital Ste-Justine

- 09h00** **Mot de bienvenue**
- 09h10** Dr. Jean-Daniel Arbour
Titre : Nouvelles approches thérapeutiques pour la DMLA.
- 09h45** A.Polosa et al.
Titre : Postnatal Hyperoxia In Rats: mfVEPs Uncover Islands Of Normal And Abnormal Visual Function.
- 10h00** Julie Lachapelle et al.
Titre : Deficits in complex information processing after mild traumatic brain injury: electrophysiological markers and vocational outcome prognosis.
- 10h15** Bupe Mwaikambo et al.
Titre : Hypoxic Regulation of the Retinal CD36 Gene.
- 10h30** **Pause café**
- 10h45** Dr. Christian Salesse
Titre : Comment la biophysique permet de comprendre l'organisation membranaire de protéines des photorécepteurs et du cycle visuel.
- 11h20** Marc-André Laurin et al
Titre : Implication des phospholipases A2 dans la phagocytose des photorécepteurs par l'épithélium pigmentaire rétinien.
- 11h35** P.Sapieha et al
Titre: Retinal Ganglion Cells Regulate Angiogenesis via Succinate and GPR91.
- 11h50** Ewelina Zimak et al
Titre: Irreversible increases in ERG amplitude and retinal thickness following postnatal exposure of Long Evans rats to bright lights.
- 12h05** Marie-Lou Garon et al
Titre : Analysis of the photopic hill : testing the glasgow model.
- 12h20** **LUNCH**
- 14h00** Réunion de l'axe Rétine
- 15h00** Levée de la Réunion

**Postnatal Hyperoxia In Rats:
mfVEPs Uncover Islands Of Normal And Abnormal Visual Function**

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Abstract:

Purpose: Previous studies have shown that postnatal hyperoxic exposure causes a severe pan-retinal disorder with retinal areas of normalcy (mfERGs). The purpose of this study was to determine whether a similar mosaic could be observed at the visual cortex.

Methods: Newborn Sprague Dawley (SD) and Long Evans (LE) rats were exposed (from P0-6, P6-14, and P0-14) to 80% O₂ and compared to rats raised in normoxia. mfERGs and mfVEPs were recorded quasi-simultaneously at P60 [VERIS 4.8; camera display unit, 1 and 37 hexagon (hx) matrices; white: 200cd.m⁻²; black: 0cd.m⁻²; background: 100cd.m⁻²; bandwidth: 10-100 Hz] with 4-minute m-sequences (interframe interval: 13.3ms). Data analysis was limited to the first order kernel.

Results: mfERG summed responses of the 37hx showed significant (p<.05) amplitude attenuations for P6-14 and P0-14 exposures [49±27% and 78±55% (SD) and 76±6% and 59±21% (LE) of control respectively]. The most prominent component of the mfVEP response (P₃) also showed a significant (p<.05) decrease in amplitude following P6-14 and P0-14 exposures to 55±43% and 45±6% (SD) and 74±44% and 57±48% (LE) of control, respectively. Analysis of individual mfVEP responses revealed that the anomalies involved mostly the superior hx of the mfVEP stimulus in SD rats, while in LE rats they were equally distributed across the entire array. In comparison, individual mfERG responses evoked from the central and inferior retinas were most affected in LE and SD rats. Of interest, a strong correlation was found between the mfERG responses (1 and 37hx) and the mfVEP P₃ value obtained with the same matrices (SD: r²: 0.64 and 0.88 and LE: r²: 0.89 and 0.83, respectively).

Conclusions: Our results thus strongly suggest that the retinopathy that results from postnatal exposure to a hyperoxic environment causes severe and (presumably) irreversible cortical damages that appear to be distributed in a fashion that could suggest a retinotopic organisation. However it remains to be determined how much of the cortical effect we report can be accounted for by the retinopathy. These anomalies are most severe in the LE rat model a finding that points once more to the increased susceptibility of this strain for postnatal oxidative stresses.

Deficits in complex information processing after mild traumatic brain injury: electrophysiological markers and vocational outcome prognosis

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There is increasing evidence that even milder forms of traumatic brain injury (MTBI) can result in persisting information processing deficits. However, the exact mechanisms involved and their relationship with global outcome remain unclear. The purpose of this study was to evaluate low-level to complex information processing using visual electrophysiology, and to examine the latter's prognostic value in regards to vocational outcome. We recorded event-related potentials (ERPs) to pattern-reversal, simple motion, texture segregation and cognitive *oddball* paradigms from 22 symptomatic mild (n=17) or moderate (n=5) TBI participants at onset of specialized clinical intervention, and from 15 normal controls. MTBI subjects presented significantly reduced amplitudes for cognitive ERPs, delayed latencies for texture and cognitive paradigms, and slower reaction times compared to controls. Cognitive symptoms were moderately correlated with cognitive ERP latency. In regards to return to work status at end of interventions, overall percentage of MTBI cases correctly classified on the basis of electrophysiological abnormalities was 77.4%. Our results indicate that individuals with symptomatic MTBI present selective deficits in complex visual information processing, with spared initial sensory input processing. ERP paradigms such as those employed in this study can reveal deficits with remain silent on traditional neuroradiological and neuropsychological evaluation, and show potential for evaluating global outcome prognosis, as well as orienting treatment and assessing cerebral recovery.

Hypoxic Regulation of the Retinal CD36 Gene

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Background: Retinal hypoxia elicits an inflammatory response that triggers membrane lipid peroxidation and subsequent generation of oxidized lipids which are prominent ligands of the CD36 scavenger receptor. Of relevance, we recently reported that inflammatory corneal neovascularization induces elevated levels of CD36. Given that CD36 is highly expressed by retinal pigment epithelial cells (RPE) and that inflammation and hypoxia occur concomitantly, we hypothesized that hypoxia modulates CD36 expression and function.

Methods and Results: Quantitative real time RT-PCR (qRT-PCR) analysis of retinal tissue from hypoxia exposed mice (8% O₂, 6h) revealed a 6-fold increase in CD36 mRNA. Subsequent experiments on human RPE indicated that hypoxia (2% O₂) induced a time-dependent increase in CD36 mRNA, protein, and surface expression. Using specific inhibitors of mRNA transcription (actinomycin D) and translation (cycloheximide), we demonstrated that the hypoxia-induced CD36 expression was a consequence of increased protein synthesis, but not elevated mRNA or protein stability. Consistent with this, inhibitors of the phosphatidylinositol-3-kinase (PI3K) pathway (wortmannin, LY294002, rapamycin), a known translational regulator, abolished the CD36 hypoxic signal. The mechanisms regulating CD36 under hypoxia were further characterized using inhibitors of the major cellular sources of reactive oxygen species (ROS). Blockers of NADPH oxidase (apocynin), lipid peroxidation (U74389G, tempol), and the mitochondrial electron transport chain (rotenone, myxothiazol) significantly attenuated the hypoxia-induced CD36 mRNA levels. Intriguingly, the functional ramifications of CD36 overexpression during hypoxia were evinced by CD36 specific augmentations in DiI-oxidized LDL internalization and apoptotic cell phagocytosis.

Conclusions: The current findings provide unique insight into a previously undisclosed induction of CD36 by hypoxia through mechanisms implicating mRNA translation, ROS generation, PI3K activation, and associated increases in CD36 scavenging activity. As such, CD36 may be critical for protecting the susceptible retina against further inflammatory injury under hypoxic conditions.

Implication des phospholipases A2 dans la phagocytose des photorécepteurs par l'épithélium pigmentaire rétinien

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Introduction : La phagocytose des segments externes des photorécepteurs (SEP) par les cellules de l'épithélium pigmentaire rétinien (EPR) est un processus crucial au maintien de l'intégrité de la rétine. Chez l'humain, plusieurs protéines ont été identifiées comme étant nécessaires au phénomène de phagocytose, mais aucune n'explique le mécanisme dans son intégrité. Plusieurs évidences suggèrent qu'une baisse du processus phagocytaire pourrait entraîner des pathologies visuelles comme la dégénérescence maculaire. Certaines homologues existent entre le processus phagocytaire immunitaire et celui de l'EPR. Nous avons déterminé précédemment que les cellules d'EPR sécrètent deux isoformes de phospholipases A2 sécrétées (sPLA2). Ces enzymes sont également impliquées dans le processus d'endocytose chez les macrophages en liant un récepteur de sPLA2 de 180 kD. **Objectifs :** Étant donné qu'il existe de très grandes similitudes entre les processus d'endocytose et de phagocytose à l'échelle cellulaire, l'objectif de ces travaux de recherche était de déterminer si certaines sPLA2 sont impliquées dans la phagocytose des SEP par l'EPR et, le cas échéant, par quel(s) mécanisme(s). **Méthodes :** Des analyses par RT-PCR et immunohistochimie ont été menées pour évaluer l'expression du récepteur de sPLA2 de 180 kD et des différentes sPLA2 par l'EPR et la lignée cellulaire ARPE-19. De plus, des lignées ARPE-19 surexprimant les sPLA2 de types IIA (formes sauvage et mutées) et V ont été générées par transfections stables de plasmides. La quantification de la phagocytose a été réalisée en mesurant l'incorporation des SEP bovins (marqués à la fluorescéine) par les cellules d'EPR en culture. Les expériences de phagocytose ont aussi été conduites en liant, à l'aide d'anticorps, les sPLA2 exprimées par l'EPR et en introduisant des sPLA2 exogènes (types IB et IA) connues, respectivement, pour lier ou non le récepteur des sPLA2 de 180 kD. **Résultats :** Le récepteur de sPLA2 de 180 kD est fortement exprimé dans l'EPR et la lignée cellulaire ARPE-19, de même que les sPLA2 de types IIA et V. Les lignées d'ARPE-19 surexprimant les sPLA2 de types IIA (forme mutée) et V ont montré une augmentation de leur capacité phagocytaire comparable à celle mesurée lors de l'addition de sPLA2 du type IB. L'ajout de sPLA2 de type IA n'a pas eu d'effet sur la capacité phagocytaire. Enfin, l'anticorps dirigé contre la sPLA2 du type IIA n'a pas eu d'effet sur la phagocytose, alors que celui dirigé contre le type V a diminué significativement celle-ci. **Conclusions :** Ces résultats suggèrent que la phagocytose des SEP par l'EPR pourrait être influencée par les sPLA2. Ces sPLA2 (IB, IIA, V) sont connues pour lier le récepteur de sPLA2 de 180 kD qui est exprimé par les cellules d'EPR. De plus, la sPLA2 de type IA, qui ne lie pas le récepteur, ne semble pas avoir d'influence sur la phagocytose. Ces données suggèrent que les sPLA2 participent à la phagocytose des SEP par l'EPR en interagissant avec le récepteur des sPLA2 de 180 kD et ce, indépendamment de leur activité enzymatique sur les membranes.

Retinal Ganglion Cells Regulate Angiogenesis via Succinate and GPR91

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Purpose: The elevated metabolic demand of neurons such as retinal ganglion cells (RGCs) is supplied by retinal vessels that form (in both physiological and pathological contexts) following the release of hypoxia-triggered pro-angiogenic factors. Although Krebs cycle intermediates such as succinate have long been known to accrue in hypoxic-ischemic states, their contribution to angiogenesis remains ill defined. Given the recent identification of a specific G-protein coupled receptor for succinate (GPR91) as well as our detection of this receptor on RGCs, **we investigated the propensity of RGCs to regulate vessel growth via a mechanism involving GPR91 and succinate.** **Methods:** Endogenous expression of retinal GPR91 was examined in Sprague-Dawley rat retinas by western blot and by immunohistochemistry in radial cryosections retrogradely labeled with Fluorogold. The RGC-5 cell line (terminally differentiated with 1 μ M of staurosporine) served as an *ex-vivo* model of RGCs. The ability of RGCs to promote vessel sprouting was determined in aortic rings co-cultured with either RGC-5 or RGC-5 conditioned media (with or without succinate treatment). Succinate-induced production of selected pro-angiogenic factors (VEGF, Angiopoietin I & II and PDGF-A) was assessed by real-time PCR in RGC-5 exposed to 100 μ M of succinate for 12 hours. **Results:** GPR91 was robustly expressed in RGCs as confirmed by co-labeling with Fluorogold positive cells. Aortic rings treated with media from RGCs or co-cultured with these neurons showed a significant increase in vessel sprouting. These effects were considerably more pronounced when RGCs were primed with succinate. In line with these findings, we observe that RGCs increase their production of VEGF, Angiopoietin I & II and PDGF-A mRNA following exposure to succinate. **Conclusions:** Our data point to a mechanism where in a time of energy mismatch, RGCs, prompted by Krebs cycle intermediates such as succinate, release pro-angiogenic factors. These neurons therefore participate in the recruitment of blood vessels in order to reinstate a metabolic equilibrium.

Irreversible increases in ERG amplitude and retinal thickness following postnatal exposure of Long Evans rats to bright lights.

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Purpose: In a previous study, we showed that compared to neonatal albino Sprague Dawley (SD) rats, retinal structure and function of pigmented Long Evans (LE) rats were significantly more damaged following postnatal hyperoxia, a finding that we attributed to the suggested pro-oxidant effect of melanin. The purpose of the present study was to further investigate the role of melanin with the use of another oxidative stress namely, bright light. **Methods:** Scotopic (intensity: -6.3 to $0.6 \log \text{ cd.m}^{-2}.\text{sec}$; 12 hrs dark adaptation) and photopic (intensity: $0.9 \log \text{ cd.m}^{-2}.\text{sec}$; background: 30 cd.m^{-2}) ERGs were recorded at P60 from juvenile SD ($n=17$) and LE ($n=8$) rats that had been exposed to a bright cyclic light (12D: 12L; 10 000 lux) from P14 to P28. Retinal histology was also performed at P60. **Results:** In SD rats, bright light exposure produced a significant decrease ($p<.05$) in amplitudes of all ERG parameters: rod Vmax (32%), rod-cone a-wave (69%), rod-cone b-wave (34%) and cone b-wave (29%) compared to controls. Similarly, retinal histology revealed a significant ($p<.05$) decrease in thickness of the photoreceptor layer (PL: 49%), the outer nuclear layer (ONL: 56%) and the outer plexiform layer (OPL:65%) while the thickness of the inner nuclear layer increased significantly (INL:72%). In contrast, LE rats showed a significant increase ($p>.05$) in all ERG parameters (rod Vmax: 42%; rod-cone a-wave: 90%; rod-cone b-wave:115% and cone b-wave: 145%) compared to normals. Similarly, retinal histology also revealed significant ($p<.05$) increases in thickness (ONL: 15%; INL: 15% and IPL: 10%) along with a reduction of the PL (41%) and OPL (29%) was noted. **Conclusions:** Clearly, the above differences between LE and SD cannot be solely attributed to the role of melanin, that is of course unless its role changes (pro-oxidant or free radical scavenger) with the source of free radicals and/or the proximity between the melanin pigment and the cellular target of reactive oxygen species produced by the oxidant. Alternatively one could also postulate that bright light exposure triggered (in LE but not in SD) a significant upregulation of retinal neurotrophic factors that prevented the normal apoptosis process thus explaining the increased retinal thickness and ERG amplitude. The latter hypothesis remains however to be tested.

ANALYSIS OF THE PHOTOPIC HILL : TESTING THE GLASGOW MODEL

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Purpose: In response to progressively brighter stimuli, the amplitude of the photopic ERG b-wave first increases to a maximal value and then gradually decreases to finally form a plateau, a phenomenon known as the Photopic Hill (PH). A mathematical model combining a Gaussian and a logistic growth function was developed by the Glasgow team to fit this unusual intensity-response curve. This model can be defined with 5 distinct parameters. We examined if these parameters could help us differentiate PHs obtained from patients affected with CSNB, cone dystrophy as well as those obtained from normal subjects during the photopic light adaptation effect. **Method:** PHs (LKC UTAS E-3000 system, background: 30 cd.m⁻²; intensities: -0.8 to 2.84 log cd.sec.m⁻²) were obtained from 3 CSNB and 4 cone dystrophy patients and 10 normal subjects. For the 10 normal subjects, photopic ERGs were obtained prior and 0, 5 and 10 minutes following a 30 minute period of dark adaptation. The parameters obtained after the fit of these curves were compared to standard parameters calculated from a representative sample of 40 normal subjects using z-score statistics. **Results:** PHs obtained from CSNB patients were best fitted using only the Gaussian function, whereas those from the cone dystrophy patients showed a significant reduction of this function ($p < 0.05$), but a normal logistic growth function ($p > 0.05$). Also, our results showed in normal subjects a significant ($p < 0.05$) time-related increase of the Gaussian function as the retina light adapts, with a non-significant ($p > 0.05$) modulation of the logistic growth function. **Conclusion:** Our results clearly indicate that the human PH can be explained by the concomitant effect of two distinct phenomena each described by its own equation, thus supporting the Glasgow model. Given the fact that CSNB presents an abolition of the ON retinal pathway while our cone dystrophy patients presumably exhibit a defect in the OFF retinal pathway, our results would suggest that the Gaussian function reflects the contribution of the OFF pathway whereas the logistic growth is more likely the result of the ON pathway activation. This being said, our results would also confirm our initial impression that the light adaptation effect most probably results from a gradual release of inhibition of the OFF pathway contribution to the ERG response enhanced by the preceding period of dark-adaptation.