

# Proceedings Meeting of the „Experimentele en Klinische Neurowetenschappen” (E.K.N.) Gent, 20<sup>th</sup> September 2002

---

**Anatomically standardized <sup>99m</sup>Tc-ECD brain perfusion SPECT allows accurate differentiation between Multi-System Atrophy and Idiopathic Parkinson’s disease.** T. BOSMAN<sup>1</sup>, K. J. R. VAN LAERE<sup>1</sup>, P. SANTENS<sup>2</sup>, J. DE REUCK<sup>2</sup>, R. A. DIERCKX<sup>1</sup> (<sup>1</sup>Division of Nuclear Medicine, Ghent University Hospital, Ghent, Belgium, <sup>2</sup>Department of Neurology, Ghent University Hospital, Ghent, Belgium).

*Introduction* : The clinical differentiation between typical idiopathic parkinson disease (IPD) and atypical parkinsonian disorders such as multiple system atrophy (MSA) is complicated by the presence of signs and symptoms common to both forms. The goal of this study was to re-evaluate the contribution of brain perfusion SPECT with anatomical standardisation and automated analysis and in the differentiation of IPD and MSA. This was performed by discriminant analysis in comparison to a large set of age- and gender-matched healthy volunteers.

*Patient and Methods* : <sup>99m</sup>Tc-ethyl cysteine dimer (ECD) SPECT was performed on 140 subjects : 81 IPD patients (age 62.6 ± 10.2 y ; disease duration 11.0 ± 6.4 y ; 50 M/31F), 15 MSA patients (61.5 ± 9.2 y ; disease duration 3.0 ± 2.2 y ; 9M/6F) and 44 age- en gender-matched healthy volunteers (age 59.2 ± 11.9 y ; 27M/17F). Patients were matched for symptom severity (Hoehn and Yahr stage). Automated predefined volume-of-interest analysis was carried out after anatomical standardisation. Stepwise discriminant analysis with cross-validation using the leave-one-out method was used to determine the subgroup of variables giving the highest accuracy for this differential diagnosis.

*Results* : Between MSA and IPD, the only regions with highly significant uptake differences after Bonferroni correction were the putamen voxel of interest (VOI). Comparing MSA versus normals and IPD, with putamen VOI values as discriminating variables, cross-validated performance showed correct classification of MSA patients with a sensitivity of 73.3% and a specificity of 84%, accuracy 83.6%. Additional input from right caudate head, left prefrontal and left mesial temporal cortex allowed 100% discrimination also after cross-validation. Discriminating between the IPD group and healthy volunteers was accurate in 94% of the cases after cross-validation, with a 91.4% sensitivity and specificity of 100%. The three-group classification (MSA, IPD and healthy volunteers), resulted in an overall accuracy of 86% post-hoc, with 98% of normals, 78% IPI and 93% of MSA correctly classified. These values were slightly lower after cross-validation : 96% for healthy volunteers, 77% for IPD and 67% for MSA (67%).

*Conclusion* : Using a large set of age- and gender-matched healthy volunteer data and anatomical standardisation, it is possible to differentiate between idiopathic Parkinson’s disease and multiple system atrophy in clinically relevant circumstances with high discriminating power.

---

**Statistical Parametric Mapping using <sup>99m</sup>Tc-ECD SPECT shows the neural networks involved in the pathophysiology of Idiopathic Parkinson’s disease and Multi-System Atrophy, correlated to clinical parameters.** T. BOSMAN<sup>1</sup>, K. J. R. VAN LAERE<sup>1</sup>, P. SANTENS<sup>2</sup>, R. A. DIERCKX<sup>1</sup> (<sup>1</sup>Division of Nuclear Medicine, Ghent University Hospital, Ghent, Belgium, <sup>2</sup>Department of Neurology, Ghent University Hospital, Ghent, Belgium).

*Introduction* : Differences in regional cerebral blood flow (rCBF) between subjects with idiopathic Parkinson’s disease (IPD) and multi-system atrophy (MSA) were investigated using statistical parametric mapping (SPM99). Comparison was made to normal volunteers and a covariance analysis with disease-specific parameters and clinical patient data was performed.

*Patient and Methods* : <sup>99m</sup>Tc-ethyl cysteine dimer (ECD) SPECT was acquired in 81 IPD patients (age 62.6 ± 10.2 y ; disease duration 11 ± 6.4 y ; 50M/31F), 15 MSA patients (age 61.5 ± 9.2 y ; disease duration 3.0 ± 2.2 y ; 9M/6F) and 44 age- and gender-matched healthy volunteers (age 59.2 ± 11.9 y ; 27M/17F). The average Hoehn and Yahr progression stage was the same 2.4 ± 1.1 both for IPD and MSA groups. All data were anatomically standardized and a group comparison was performed by SPM99. In addition, covariate analysis was undertaken on the images taking disease duration, medication and clinical subscores as variables.

*Results* : Applying a height threshold of  $p < 0.05$  corrected, significant hypoperfusion in IPD was present compared to normals in the bilateral basal ganglia, thalami, prefrontal and lateral frontal cortex, as well as in the parieto-occipital cortex bilaterally. For MSA, only symmetric hypoperfusion clusters in the putamen and thalamus were found with respect to normals, in addition to a localized cluster in the lateral frontal cortex. Compared to normals, relative hyperperfusion was similar for both MSA and IPD in the vermis and bilateral upper cerebellar hemisphere. When comparing IPD and MSA directly, a relative hypoperfusion of the bilateral posterior putamen and mesencephalon was found for MSA. Covariate analysis ( $p < 0.001$  uncorrected) showed a negative correlation between perfusion of the caudate heads and limbic system and the standardized dosage of (non-levodopa) dopamine agonists in the parkinsonian patients (uncorrected  $p < 0.001$ ), while for MSA a bilateral decrease in putamen rCBF was noted. Cognitive changes were correlated to limbic and parieto-occipital systems rather than dysexecutive prefrontal regions.

*Conclusion* : The regional perfusion pattern for Parkinson's disease corresponds to a symmetrical widespread sub-cortical and neocortical network involved in its pathophysiology. The typical feature of MSA is a posterior putamen hypoperfusion, which can be used for differential diagnosis by rCBF imaging studies. Dopamine agonists have a different effect between MSA and IPD. Cognitive changes in IPD or MSA are correlated to limbic and parieto-occipital dysfunction rather than prefrontal changes.

**Reevaluation of intraperitoneal injection of pentylenetetrazol as a model for generalized epilepsy using digital video-EEG monitoring.** P. CLAEYS, K. VRONCK, S. DEDEURWAERDERE, P. BOON (Reference Center for Refractory Epilepsy, Department of Neurology, Ghent University Hospital, Ghent, Belgium).

*Introduction* : Intraperitoneal injection of Pentylenetetrazol (PTZ-i.p.) in rats causes generalized seizure activity ranging from absences to generalized tonic clonic seizures. PTZ is often used as a screening method for testing the efficacy of new antiepileptic therapies. In the literature different rat strains are used and reported PTZ dosages are variable and range from 25 to 125 mg/kg. The electroclinical correlation in reported experiments was not assessed by video-EEG monitoring. The purpose of our study is to reevaluate PTZ-i.p. (45 mg/kg) in two different commonly used rat strains in video-EEG monitoring conditions.

*Methods* : 9 male Wistar (WI) and 11 male Sprague-Dawley (SD) rats were implanted with 5 subdural EEG electrodes. During continuous digital video-EEG monitoring the rats were injected with 45 mg/kg PTZ-i.p. The electroclinical correlation of ictal events was documented by means of the following parameters within 60 min post-injection : main clinical seizure type ; number of seizures ; duration of seizures ; time to (first) seizure(s). Results were compared between WI and SD.

*Results* : For both strains the following seizure types (t), the mean number of seizures (n), the mean duration per seizure (d), and the mean time to first seizure (x) were documented : typical absences (WI : t = 0 ; n = 0 // SD : t = 1 ; n = 77 ; d = 2,3s ; x = 925s) ; isolated myoclonic jerks (WI : t = 0 ; n = 0 // SD : t = 1 ; n = 55 ; d = 1s ; x = 86s), major motor events resembling tonic clonic seizures (WI : t = 8 ; n = 1 ; d = 31,5s ; x = 74s // SD : t = 8 ; n = 1 ; d = 33,9s ; x = 94s) and generalized tonic clonic status epilepticus (WI : t = 1 ; n = 1 ; d = 292s ; x = 59s // SD : t = 1 ; n = 1 ; d = 365s ; x = 60s). In all animals of both strains, major motor events were consistently followed by isolated myoclonic jerks and/or absences.

*Conclusions* : Video-EEG analysis of clinical characteristics, frequency, duration and time course of PTZ-i.p. induced seizures at a dose of 45 mg/kg showed no significant differences in Wistar rats versus Sprague-Dawley rats.

Supported by grants from the Fund for Scientific Research-Flanders (F.W.O.), Ghent University Research Fund (B.O.F.) and the Clinical Epilepsy Grant Ghent University Hospital 1998-2001.

**Hippocampal dopaminergic and serotonergic involvement in the anticonvulsant mechanism of action of oxcarbazepine and 10, 11-dihydro-10-hydroxycarbamazepine.** R. CLINCKERS<sup>1</sup>, I. SMOLDERS<sup>1</sup>, A. MEURS<sup>2</sup>, G. EBINGER<sup>2</sup>, Y. MICHOTTE<sup>1</sup> (<sup>1</sup>Pharmaceutical Chemistry and Drug Analysis, Experimental Neuropharmacology, Vrije Universiteit Brussel, Brussels, Belgium, <sup>2</sup>Neurology, University Hospital AZ-VUB, Brussels, Belgium).

*Introduction* : The present microdialysis study aimed at evaluating the anticonvulsant role of hippocampal serotonin 5-HT<sub>1A</sub> and dopamine D<sub>2</sub> receptor activation in focally evoked pilocarpine-induced seizures in rats, with concomitant assessment of the possible reciprocal interaction between hippocampal dopamine (DA) and serotonin (5-HT). We further investigated the monoaminergic involvement in the anticonvulsant mechanism of action of oxcarbazepine (OXC), and its active metabolite 10,11-dihydro-10-hydroxycarbamazepine (MHC). The effects of intrahippocampally applied 5-HT and DA, monoamine re-uptake blockers (fluoxetine and GBR-12909), OXC and MHC alone and in combination with a selective 5HT<sub>1A</sub> or D<sub>2</sub> blocker (WAY 100635 and remoxipride) were evaluated.

*Animals and Methods* : Twenty-four hours after stereotactic probe implantation in the hippocampus, microdialysis experiments, with electrocorticographic monitoring, were performed on conscious male albino Wistar rats (260-300 g), according to previously described procedures (1). Pilocarpine, a muscarinic agonist, was perfused through the probe (10 mM, 40 min at 2  $\mu$ l/min) to evoke limbic seizures. Microbore chromatographic conditions and procedures for 5-HT and DA analysis have previously been described in detail (2).

*Results* : Only a specific 5-HT (0.6-1.2 nM) or DA (0.5-1 nM) concentration range protected animals from pilocarpine-induced seizures. Dose-response experiments were carried out to determine the anticonvulsant threshold doses of fluoxetine, GBR 12909, OXC and MHC. All selected threshold doses (fluoxetine : 5  $\mu$ M, GBR 12909 : 100  $\mu$ M, OXC : 100  $\mu$ M, MHC : 250  $\mu$ M) provoked hippocampal 5-HT and/or DA increases that were within the 5-HT and DA protective concentration ranges described above. Co-perfusion of these threshold doses with WAY 100635 (100 nM) or remoxipride (4  $\mu$ M) abolished the anticonvulsant effects : all animals exhibited clear limbic seizures. There was a marked similarity between the monoamine increases induced by perfusion of the re-uptake blockers and the anticonvulsants, OXC and MHC. Finally, no bi-directional interaction between DA and 5-HT was observed when 5-HT, DA or the studied drugs were perfused in selective concentrations.

*Conclusion* : In contrast to the substantia nigra (3, 4), hippocampal 5-HT-ergic and DA-ergic systems do not exhibit a reciprocal interaction. Both transmitter systems contributed independently to the prevention of hippocampal epileptogenesis via 5-HT<sub>1A</sub> and D<sub>2</sub> receptor activation. In view of the structural similarity with the tricyclic antidepressants, and the presently determined similar increases in hippocampal extracellular levels of 5-HT and DA, we suggest that an interaction of OXC and MHC with the monoamine re-uptake sites is likely to occur.

The hypothesis about this potential mechanism of action needs to be confirmed by receptor binding studies.

### References

1. Smolders I. *et al.* Effectiveness of vigabatrin against focally evoked pilocarpine-induced seizures and concomitant changes in extracellular hippocampal and cerebellar glutamate,  $\alpha$ -aminobutyric acid and dopamine levels, a microdialysis-electrocorticography study in freely moving rats. *The Journal of Pharmacology and Experimental Therapeutics*, 1997, **283** : 1239-1248.
2. Sarre S. *et al.* Microbore liquid chromatography analysis of monoamine transmitters. *Methods in Molecular Biology*, 1997, **72** : 185-96.
3. Thorré K. *et al.* 5-HT<sub>4</sub> receptor involvement in the serotonin-enhanced dopamine efflux from the substantia nigra of the freely moving rat : a microdialysis study. *Brain Research*, 1998, **796** (1- 2) : 117-24.
4. Thorré *et al.* Dopaminergic regulation of serotonin release in the substantia nigra of the freely moving rat using microdialysis. *Brain Research*, 1998, **796** (1-2) : 107-16.

---

**Single subject hippocampal activation detected with fMRI : preliminary results.** K. DEBLAERE<sup>1</sup>, A. TIELEMAN<sup>1</sup>, P. VANDEMAELE<sup>1</sup>, K. VONCK<sup>2</sup>, G. VINGERHOETS<sup>3</sup>, P. BOON<sup>2</sup>, E. ACHTEN<sup>1</sup> (<sup>1</sup>Department of Neuroradiology, Ghent University Hospital, Ghent, Belgium, <sup>2</sup>Reference Center for Refractory Epilepsy, Department of Neurology, Ghent University Hospital, Ghent, Belgium, <sup>3</sup>Laboratory for Neuropsychology, Department of Neurology, Ghent University Hospital, Ghent Belgium).

*Introduction* : Multiple fMRI studies have demonstrated that the hippocampus is essential in memory formation (1). However, so far no study has reported reliable single subject hippocampal activation with fMRI. This would be very useful for clinical use, for instance in the pre-surgical evaluation of patients with pharmacoresistant temporal lobe epilepsy (TLE) considered for anterior temporal lobectomy. Recently, Moritz *et al.* suggested the use of a variable length block design to elicit single subject hippocampal activation, combining the accuracy of event-related fMRI and the power of a simple blocked design. The purpose of this study was to assess the reproducibility of hippocampal activation in healthy subjects using this paradigm. Preliminary results in two left TLE patients are also reported.

*Subjects and methods* : So far, six healthy subjects (2 male, 4 female) and two left TLE patients (both female) participated in this study. Before and during scanning of an anatomical scan, the subjects viewed 5 images of the picture set of Snottgrass and Vanderwart. Following the anatomical scan, two runs of 260 images were acquired while subjects viewed variable length blocks (2, 3) of new images (NEW) mixed with variable blocks of the images seen before (OLD). Subjects made the decision OLD/NEW with button presses.

The contrast OLD versus NEW (1) was calculated using the software package SPM99 and individual statistical results were thresholded at  $p < 0.05$ .

*Results* : All subjects had strong activation foci in the mesial temporal lobe bilaterally, especially in parahippocampal gyrus and anterior fusiform gyrus. Hippocampal activation foci were present in 5/6 subjects, without clear lateralization. The two patients both showed clearly right lateralized mesiotemporal activation patterns, confirming the results of the intracarotid amyltal test.

*Conclusion* : Both results in healthy subjects and TLE patients are very promising and larger subject and patient samples will assess the clinical value of this fMRI paradigm.

### References

1. Stern C. E., Corkin S., Ciliberto González R., Guimaraes A. R., Baker J. R., Jennings P. J., Carr C. A., Sugiura R. M., Vedantham V., Rosen B. R. The hippocampal formation participates in novel picture encoding : evidence from functional magnetic resonance imaging. *Proc. Natl. Acad. Sci. USA*, 1996, **93** : 8660-8665.
2. Liu T. T., Frank L. P., Wong E. C., Buxton R. B. Detection power, Estimation efficiency, an predictability in event-related fMRI. *Neuroimage*, 2001, **13** : 759-773.
3. Moritz C. H., Johnson S. J., Meyerand M. E. Variable-length block memory encoding paradigm demonstrates individual subject hippocampal fMRI response. *Proc. Intl. Soc. Magn. Res. Med.*, 2002, 10 (abstract).

---

**Vagus nerve stimulation in Genetic Absence Epilepsy Rats from Strasbourg (GAERS).** S. DEDEURWAERDERE<sup>1</sup>, K. VONCK<sup>1</sup>, M. D'HAVÉ<sup>1</sup>, D. NARITOKU<sup>2</sup>, T. GRISAR<sup>3</sup>, P. BOON<sup>1</sup> (<sup>1</sup>Reference Center for Refractory Epilepsy, Department of Neurology, Ghent University Hospital, Ghent, Belgium, <sup>2</sup>Department of Neurology, Southern Illinois University, Illinois, U.S.A., <sup>3</sup>Department of Neurobiology, University of Liege, Liege, Belgium).

*Introduction* : Vagus nerve stimulation (VNS) is a neurophysiological treatment for partial seizures with or without secondary generalization. In a previous study acute VNS had no significant influence on the number, duration and frequency of spike and wave discharges (SWD) in GAERS. The effect of chronic VNS in GAERS has not been studied yet. The aim of the present work is to evaluate chronic VNS in this model of primary generalized epilepsy.

*Methods* : Nineteen GAERS were implanted with 5 epidural EEG electrodes and a stimulation electrode around the left vagus nerve. Subsequently they underwent long-term video-EEG monitoring during a 2-week period. Between day 1 and day 7 (baseline EEG recording) the animals were not stimulated. After baseline EEG recording, 10 GAERS were stimulated 24 hours-a-day from day 8 until day 14 with the following stimulation parameters : output current : 1.5 mA, frequency : 30 Hz, pulse width : 500  $\mu$ sec, on/off time : 60 sec/12 sec. Nine control GAERS underwent implantation of recording and stimulation electrodes but were not stimulated. The number, duration and frequency of SWD were compared between day 7 and day 14.

*Results* : In stimulated GAERS, average number per hour, duration and frequency of SWD in baseline conditions (day 7) was 23 (SD = 20.1), 11 sec (SD = 5.6) and 8.0 Hz (SD = 0.45) respectively. During the last day of VNS (day 14) the average number of SWD per hour, duration and frequency was 27 (SD = 21.6), 9.3 sec (SD = 4.3) and 7.9 Hz (SD = 0.38). In baseline conditions (day 7), control rats had an average number of SWD per hour of 35 (SD = 34.4). The average duration and frequency of SWD were 12 sec (SD = 6.4) and 8.1 Hz (SD = 0.49) respectively. During day 14, these figures were 20 SWD (SD = 15.9) per hour, 11 sec (SD = 6.4) and 7.7 Hz (SD = 0.52). When control GAERS versus stimulated GAERS, and baseline conditions (day 7) versus stimulation conditions (day 14) were compared, no statistically significant differences could be found.

*Conclusion* : VNS according to a 7-day/24-hours-per-day-protocol, has no significant influence on the number, duration and frequency of SWD in GAERS. Further studies using other stimulation parameters should confirm that VNS is not active in this model of primary generalized epilepsy.

---

**Measuring synchrony and similarity for anticipating epileptic seizures : a case study.** E. GYSELS<sup>1,2</sup>, P. DE SOMERE<sup>1</sup>, K. VONCK<sup>1</sup>, R. VAN DE WALLE<sup>1</sup>, P. BOON<sup>2</sup>, I. LEMAHIEU<sup>1</sup> (<sup>1</sup>Department of electronics and information systems, Engineering Faculty, Ghent University Hospital, Ghent, Belgium, <sup>2</sup>Reference Center for Refractory Epilepsy, Department of Neurology, Ghent University Hospital, Ghent, Belgium).

*Introduction* : The aim of this study was to compare two methods used for anticipating epileptic seizures : the similarity index and the Phase Locking Value (PLV).

*Patient and Methods* : We considered a patient with left medial temporal lobe epilepsy. During monitoring we recorded two seizures. By studying the synchronization between different brain regions and calculating the similarity index, one seizure could be anticipated. We repeated the calculations in several frequency bands.

*Results* : In the patient with medial temporal lobe epilepsy, one out of two seizures could be anticipated with these methods. None of the methods outperforms the other one. The advantage of studying synchronization is that it tells something about the interaction between different brain regions.

The anticipation time with the similarity index was about 15 minutes. When we considered the PLV, we noticed an important loss of synchrony about two hours before the seizure. This suggests that, for this specific type of epilepsy, PLV can be used to reduce the number of false positives of the similarity method.

It is important to choose proper frequency bands : by filtering EEG in small frequency bands, we remove phenomena intervening in epileptogenesis. The detected phenomena are different for both methods, as well as the frequency bands in which they appear. However, the brain region involved seems to be the same in both cases.

*Conclusion* : So far, methods introduced for the anticipation of epileptic seizures can be used only for a specific type of epilepsy. In general, the sensitivity and specificity are very low. The comparison of the similarity index and the level synchrony suggests that different phenomena interfere, at different time scales and in different frequency bands.

---

**S 100B, S100A1, S100A2, S100A4 AND S100A6 Calcium-binding proteins in a rat model of cerebral basilar system artery vasospasm.** F. LEFRANC<sup>1</sup>, R. POCHE<sup>2</sup>, C. HEIZMANN<sup>3</sup>, C. DECAESTECKER<sup>2</sup>, J. BROTCHE, I. SALMON<sup>4</sup>, R. KISS<sup>2</sup> (<sup>1</sup>Departments of Neurosurgery and <sup>4</sup>Pathology, Erasmus University Hospital, Brussels, Belgium, <sup>2</sup>Laboratory of Histopathology, Faculty of Medicine, Free University of Brussels, Brussels, Belgium, <sup>3</sup>Division of Clinical Chemistry and Biochemistry, Department of Pediatrics, University of Zürich, Zürich, Switzerland).

*Introduction* : Subarachnoid hemorrhage (SAH) induces severe neurological morbidity and mortality. Modifications in the levels of expression of some intracellular signalling elements controlling the organization of the actin cytoskeleton (including the RhoA small GTPase and its related kinases) play significant roles in the induction of smooth muscle cell contraction, a calcium-dependent process which is pathognomic of SAH-induced vasospasm at molecular level. Several members of the calcium-binding S100 protein family are known to exercise a significant control on the organization of the actin cytoskeleton. This is why we have looked at the levels of expression of S100 proteins in SAH-induced vasospasm.

*Methods* : We therefore made use of a “double hemorrhage” rat model of SAH-induced vasospasm and investigated by immunohistochemistry if the levels of expression of S100B, S100A1, S100A2, S100A4 and S100A6 proteins were significantly modified in this pathology.

*Results* : The quantitative determination of this immunohistochemical expression (carried out by means of computer-assisted microscopy) revealed that SAH-induced vasospasm is accompanied by a very significant increase in S100B, S100A2 and, to a lesser extent in S100A4 and S100A6 expression, but not by S100A1 expression.

*Conclusion* : Such modifications of members of the S100 protein family in SAH-induced vasospasm could relate to the various roles played by this specific class of calcium-binding proteins on actin cytoskeleton organization. These modifications to S100 protein expression seem relatively specific to SAH-induced vasospasm since heparin-induced epilepsy-like symptomata were accompanied by dramatically distinct profiles of S100 protein expression.

---

**Evidence supporting the link between changes in hippocampal extracellular glutamate and GABA levels and seizure activity.** A. MEURS<sup>1</sup>, R. CLINCKERS<sup>2</sup>, I. SMOLDERS<sup>2</sup>, G. EBINGER<sup>1</sup>, Y. MICHOTTE<sup>2</sup> (<sup>1</sup>Neurology, University Hospital AZ-VUB, Brussels, Belgium, <sup>2</sup>Pharmaceutical Chemistry and Drug Analysis, Experimental Neuropharmacology, Vrije Universiteit Brussel, Brussels, Belgium).

*Introduction* : Glutamate and GABA, the main excitatory and inhibitory neurotransmitters in the CNS, are known to play a central role in the physiopathological mechanisms underlying seizure activity. We previously observed that intrahippocampal administration of the muscarinic receptor agonist pilocarpine, which produces limbic motor seizures, also leads to increases in hippocampal extracellular glutamate and GABA (1). However, the link between these changes and seizure activity has been disputed in literature (2). In this study, we investigated the behavioural, electrographic and neurochemical changes that occurred after intrahippocampal administration of picrotoxin, a GABA<sub>A</sub> receptor antagonist, and compared these changes to those observed following intrahippocampal pilocarpine administration.

*Materials and Methods* : Seizures were evoked in conscious male albino Wistar rats (270-300 g) by perfusing pilocarpine (10 mM, 40 min at 2 µl/min) or picrotoxin (100 µM, 40 min at 2 µl/min) via a microdialysis probe into the hippocampus (3). Seizure activity was monitored by electrocorticography. Dialysate hippocampal glutamate and GABA levels were determined by microbore liquid chromatography (4).

*Results and Conclusions* : Intrahippocampal administration of both pilocarpine and picrotoxin produced remarkably similar behavioural and electrocorticographic changes, implying that the behavioural abnormalities which were observed during these experiments were indeed manifestations of seizure activity induced at the level of the hippocampus, and not merely the result of muscarinic stimulation or GABA<sub>A</sub> receptor blockade. Both pilocarpine and picrotoxin produced significant increases in extracellular concentrations of glutamate and GABA in hippocampus. The finding that limbic seizures, evoked by two distinct pharmacological mechanisms, result in similar changes in extracellular concentrations of glutamate and GABA strongly suggests that these changes can be attributed to seizure activity itself, and not to specific pharmacological effects of the administered compounds. However, increases in glutamate were significantly higher and lasted longer in the picrotoxin group, while increases in GABA were of similar extent but of shorter duration compared to the pilocarpine control group. We hypothesise that picrotoxin induces seizures through disinhibition of

hippocampal glutamatergic neurons, by inhibiting postsynaptic GABA<sub>A</sub> receptors. Pilocarpine, on the other hand, is thought to produce seizures through a K<sup>+</sup>-channel mediated increase in hippocampal excitability. This could explain why the increases in extracellular glutamate are higher and last longer in the picrotoxin group. We suggest that the increases in extracellular GABA after pilocarpine administration are the result of both direct muscarinic stimulation of hippocampal GABA-ergic interneurons and activation of local inhibitory feedback mechanisms.

### References

1. Smolders I. *et al.* Hippocampal and cerebellar extracellular amino acids during pilocarpine-induced seizures in freely moving rats. *European Journal of Pharmacology*, 1997, **319** : 21-29.
2. Obrenovitch T. P. High extracellular glutamate and neuronal death in neurological disorders. Cause, contribution or consequence ? *Annals of the New York Academy of Sciences*, 1999, **890** : 273-286.
3. Smolders I. *et al.* NMDA receptor-mediated pilocarpine-induced seizures : characterization in freely moving rats by microdialysis. *British Journal of Pharmacology*, 1997, **121** : 1171-1179.
4. Smolders I. *et al.* The analysis of excitatory, inhibitory and other amino acids in rat brain microdialysates using microbore liquid chromatography. *Journal of Neuroscience Methods*, 1995, **57** : 47-53.

---

**Stereotactic radiofrequency ablation of the pituitary gland in the rat : validation by means of immunohistochemical and chromatographic methods.** R. RAEDT<sup>1,2</sup>, J. CAEMAERT<sup>3</sup>, F. ÖDBERG<sup>4</sup>, F. JACOBS<sup>1</sup> (<sup>1</sup>Laboratory of Zoöphysiology, Department of Biology, Faculty of Science, Ghent University, Ghent, Belgium, <sup>2</sup>Reference Center for Refractory Epilepsy, Department of Neurology, Ghent University Hospital, Ghent, Belgium, <sup>3</sup>Department of Neurosurgery, Ghent University Hospital, Ghent, Belgium, <sup>4</sup>Department of Animal Nutrition, Genetics, Breeding and Ediology, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium).

*Introduction* : This research aimed to develop a radiofrequency approach for hypofysectomy in the rat. The elimination of the hypothalamo-pituitary-adrenal (HPA) axis was checked by evaluating the secretion and anatomical structure of the adrenal glands. Post mortem the pituitary gland was tested for its functionality.

*Methods* : An electrode, connected to a radiofrequency generator, was transauricularly positioned in the pituitary gland by means of a stereotactic apparatus (based on the method of GAY), which was built specifically for this experiment. The maximum power delivered by the generator was fixed on 8 Watt and the pituitary gland was heated up during 22 seconds to a temperature of 80°C. The disconnection of the HPA axis was verified by measuring the secretion of the adrenal glands. Thereby the relative corticosterone concentration was quantified with the HPLC method. Post mortem, the area of the adrenal glands was calculated and the sella turcica was examined for functional (hormone producing) remnants. For the latter, automatized immunohistochemistry (Ventana ES 320) was used with anti-ACTH as a primary antibody. We have also investigated whether the hypofysectomised rats behaved in a different way in a novel environment by using the open-field test and the emergence test.

*Results* : Unlike in untreated rats, corticosterone was not detectable in the blood of hypofysectomised rats, with the used accuracy. The area of the adrenal cortex of hypofysectomised rats was significantly smaller (Mann-Withney U, p = 0.049) compared to the cortex area of untreated rats. Examination of the sella turcica showed the presence of very small remnants of the pituitary gland in all hypofysectomised rats. In two of the three cases the residue was no longer functional. In the third case the remnant still produced A CTH, but evaluation of the adrenal functionality showed that the production wasn't sufficient to sustain the HPA axis.

The behavioral tests showed that novelty-induced defecation did not occur in the case of hypofysectomised rats. In the emergence test the hypofysectomised rats left the protective area more quickly to expose themselves to potential danger.

*Discussion* : The results allow us to conclude that the radiofrequency hypofysectomy technique is able to reduce the pituitary to a small, non-functional remnant. An evaluation of the peripheral consequences showed that the destruction of the pituitary gland was sufficient to set off the HPA axis. Moreover, there was no detectable corticosterone present in the blood and a significant atrophy of the adrenal cortex was achieved. On the basis of the results of the emergence test we suspect that the hypofysectomised rats are less fearful in a novel environment.

---

**A high field magnetic resonance anatomic analysis of the subthalamic nucleus.** K. RIJKERS, V. VAN DE WALLE, Y. TEMEL, L. VANORMELINGEN, M. VANDERSTEEN, P. ADRIAENSEN, J. GELAN, E. BEULS (Department of Neurosurgery of the University of Maastricht, The Netherlands, Department of Anatomy and Department of Material research of the University Of Limburg, Diepenbeek-Hasselt, Belgium).

*Introduction* : High frequency stimulation of the subthalamic nucleus (STN) is nowadays a widely performed method to treat idiopathic Parkinson's disease. Few detailed microscopic studies of this nucleus are available in literature. The aim of our study is to visualize the STN using high-field MRI scanning.

*Methods* : During postmortem investigation on a human brain a cubicle of tissue in the area around the STN was isolated. This brain tissue was scanned in the three orthogonal planes using a Varian (Varian Nuclear Magnetic Resonance Instrument, Palo Alto, CA) Unity 400 spectrometer (9.4 Tesla) with a slice thickness of 1 mm. The images generated this way were compared to photographs of conventionally stained slices of brain tissue in different neuroanatomical books.

*Results* : The STN is recognizable in the scans. In all three of the orthogonal planes it appears as a biconvex lens. The laterocaudal tip of the nucleus adjoins the rostral part of the substantia nigra. More cranially, the STN is situated mediodorsally to the cerebral peduncle. Other recognizable structures are the superior colliculus, medial and lateral geniculate bodies, red nucleus and several tracts.

*Conclusion* : High field MRI scanning is an appropriate method to visualize the microanatomy of the STN and surroundings. It provides images with an inplane resolution comparable with the optical microscope including all the advantages inherent to MR technology. The images allow an optimal analysis of the microenvironment of the STN in the three orthogonal planes.

**Lateralized effects of STN stimulation in Parkinson's disease on different characteristics of speech.** P. SANTENS, M. DE LETTER, J. VAN BORSEL (Departments of Neurology, Oto-Rhino-Laryngology, Ghent University Hospital, Ghent, Belgium).

*Introduction* : Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is increasingly accepted as an effective treatment for well-selected patients with advanced PD. Beneficial effects have been demonstrated on all cardinal features of PD. The effects on speech, however, are variable. Although some have found speech to be improved, increase of dysarthria is a serious problem in a substantial number of patients with STN stimulation.

*Methods* : Seven PD patients who had bilateral STN stimulation after careful pre-operative testing were included. DBS was performed using conventional stereotactic procedures and intra-operative macro-electrode stimulation. As expected, patients had a significant improvement of motor symptomatology. Speech testing was performed at a mean of 10 months following surgery. Patients were asked to read a standard passage during four different stimulation conditions (left on, right on, bilateral on/off). These segments were video-taped to be evaluated by 22 qualified speech therapists for six characteristics : prosody, articulation, intelligibility, quality of voice, loudness and rate. Scores were given on a visual analog scale. Non-parametric tests were used for statistical analysis.

*Results* : Significant effects of stimulation condition were found for articulation, prosody and intelligibility. Left-sided stimulation had significant negative effects on these parameters, especially when right-sided stimulation was off. Right-sided stimulation did not demonstrate this effect, there was no difference when comparing bilateral on vs. off.

*Conclusions* : Although we cannot conclude on the mechanisms involved, it seems that some aspects of speech are differentially altered by left and right STN stimulation. This might be due to imbalanced tuning between left and right basal ganglia circuitry. More importantly, this might have implications for the management of this compelling side-effect of STN stimulation.

**Presurgical functional magnetic resonance imaging : usefulness as perceived by the clinicians.** A. TIELEMAN<sup>1</sup>, K. DEBLAERE<sup>1</sup>, P. VANDEMAELE<sup>1</sup>, J. CAEMAERT<sup>2</sup>, P. BOON<sup>3</sup>, L. DEFREYNE<sup>1</sup>, E. ACHTEN<sup>1</sup> (<sup>1</sup>Department of Neuroradiology, Ghent University Hospital, Ghent, Belgium, <sup>2</sup>Department of Neurosurgery, Ghent University Hospital, Ghent, Belgium, <sup>3</sup>Reference Center for Refractory Epilepsy, Department of Neurology, Ghent University Hospital, Ghent, Belgium).

*Introduction* : The aim was to evaluate the value of fMRI on therapy management in patients with focal brain lesions.

*Patients and Methods* : The medical records of 31 consecutive patients, with focal brain lesion of different origin and referred for fMRI were reviewed. The main reason for referral was that the planned interventional procedure could result in severe functional deficit if adjacent eloquent cortex was removed or damaged during surgery. During the fMRI examination, a variety of motor and language tasks were used in die individual patient, depending upon the localization of the lesion. All data were processed using SPM99.

Proximity was considered important if functional activation was present not more than 1 gyrus away from the bordering lesion. Language dominance was important for lesions in the vicinity of the classical primary language related areas in any hemisphere. We retrospectively reviewed how often and in which way these fNM results contributed to the planning of neurosurgical procedures.

*Results* : Useful functional activation from motor and/or language tasks was present for all patients but one.

After questioning the referring clinicians, the results of the fMRI studies yielded additional clinical information in 26 of the 31 patients. In 5, the fMRI results were considered redundant to clinical or structural MRI information, but language lateralization was considered a bonus in 2 of these.

*Conclusion* : This study indicates that fMRI yields serious information to assess possible surgical risks. In only 3/31 patients, did elective fMRI not really contribute to the surgical decision making.

### References

1. Achten E., Jackson G. D., Cameron J. A., Abbott D. F., Stella D. L., Fabinyi G. C. A. Presurgical Evaluation of the Motor Hand Area with Functional MR maging in Patients with tumours and Dysplastic lesions. *Radiology*, 1999, **210** : 529-538 S.
2. Dymarkowski, Sunaert S., Van Oostende S., Van Hecke P., Wilms G., Demaerel P., Nuttin B., Plets C., Marchal G. Functional MRI of the brain : localisation of eloquent cortex in focal brain lesion therapy. *Eur. Radiol.*, 1998, **8** : 1573-1580.
3. Papke K., Hellmann T., Renger B., Morgenroth C., Knecht S., Schuierer G., Reimer P. Clinical applications of functional MRI at 1.0T : motor and language studies in healty subjects and patients. *Eur. Radiol.*, 1999, **9** (2) : 211-220.

---

**Influence of stimulus pacing on language lateralization with fMRI using a semantic decision task.** A. TIELEMAN<sup>1</sup>, K. DEBLAERE<sup>1</sup>, P. VANDEMAELE<sup>1</sup>, G. VINGERHOETS<sup>2</sup>, E. ACHTEN<sup>1</sup> (<sup>1</sup>Department of Neuroradiology, Ghent University Hospital, Ghent, Belgium, <sup>2</sup>Laboratory for Neuropsychology, Department of Neurology, Ghent University Hospital, Ghent Belgium).

*Introduction* : In this study, we examined the influence of stimulus pacing on the determination of global and regional hemispheric dominance for language using a semantic decision task in healthy subjects.

*Subjects and methods* : Until now, 6 healthy volunteers participated in this study. Two experiments were performed in which semantic categorization of words (animal or object) was contrasted with a non-semantic uppercase/lowercase discrimination (1). The words were visually presented and the decisions were made with button presses. During the first experiment a new stimulus was given every 3 seconds (fixed paced) and during the second a new stimulus appeared immediately after the subject had pressed a button (self-paced). Image postprocessing and statistical analysis was performed using the SPM99 software.

Lateralization indices (LI) were calculated for the cerebral regions as a whole, and for two regions of interest : fronto-lateral convexity (FroLat) ; and the superior and middle temporal, supramarginal and angular gyrus (TemPar).

*Results* : FroLat activation was observed in all 6 and T emPar activation in 5 studies. Determination of both fronto-lateral and temporoparietal LI resulted in more strongly left lateralized results for the self-paced task compared to the fixed-paced task. Global LI were unreliable and inconsistent during the fixed paced experiment ; during the self-paced experiment global LI were more consistent, but less powerful because of more bilateral secondary and tertiary visual regions activated.

*Conclusion* : Determination of the global LI can be misleading (3). Since productive and receptive language functions (supposed to be located more frontal and parietal respectively), are not necessarily served by the same hemisphere, lateralization should be determined for both regions separately (2). Although more study need to be performed on more subjects, we demonstrate that stimulus pacing can be of major influence in the results of fMRI language lateralization and localisation.

### References

1. Demb J. B., Desmond J. E., Wagner A. D., et al. *J. Neurosci.*, 1995, **15** :5870-5878.
2. Kurthen M, Helmstaedter c, Linke DB, et al. *Brain Lang*, 1992, **43** :694-712.
3. Benson RR, Fitzgerald DB, LeSueur LL, et al. *Neurology*, 1999, **52** : 798-809.

---

**Resuscitative mild Hypothermia reduces striatal neurotransmitter levels and histological brain damage in the endothelin-1 model for focal cerebral ischaemia.** A. VAN HEMELRIJCK<sup>1</sup>, D. VERMIJLEN<sup>2</sup>, S. HACHIMI-IDRISSI<sup>3</sup>, S. SARRE<sup>1</sup>, G. EBINGER<sup>4</sup>, Y. MICHOTTE<sup>1</sup> (<sup>1</sup>Pharmaceutical Chemistry and Drug Analysis, Experimental Neuropharmacology, Vrije Universiteit Brussel, Brussels, Belgium, <sup>2</sup>Laboratory for Cell Biology and Histology, Vrije Universiteit Brussel, Brussels, Belgium, <sup>3</sup>Department of Critical Care Medicine and Cerebral Resuscitation Research Group, AZ-VUB, Vrije Universiteit Brussel, Brussels, Belgium, <sup>4</sup>Department of Neurology, AZ-VUB, Vrije Universiteit Brussel, Brussels, Belgium).

*Introduction* : The extracellular striatal glutamate and dopamine concentrations and the concomitant ischaemic damage were studied following induction of transient focal cerebral ischaemia under normothermic (37°C) and post-ischaemic (resuscitative) mild hypothermic (34°C) conditions in sevoflurane anaesthetized male Wistar rats.

*Patient and Methods* : Microdialysis was used to sample neurotransmitters from the striatum. Focal ischaemia was induced by infusing endothelin-1 adjacent to the middle cerebral artery. Animals in one group were maintained under normothermic conditions during the whole experiment. Another group of rats was exposed to 2 h of mild hypothermia, which started with a delay of 20 min after the ischaemic insult. The brain temperature was continuously recorded by a thermocouple probe. The volume of ischaemic damage and the degree of apoptosis were determined 24 h after the insult.

*Results* : Initial increases in glutamate and dopamine following endothelin-1 infusion were not influenced by resuscitative mild hypothermia. In the hypothermic group however, extracellular glutamate levels returned to baseline levels whereas they remained enhanced in the normothermic group. Hypothermia halved the mean infarct volume, particularly due to a decreased cortical damage and reduced the amount of fragmented cortical nuclei.

*Conclusion* : these results indicate that resuscitative mild hypothermia attenuates prolonged striatal glutamate overflow and cortical apoptosis.

---