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Symposia and Free Communications

Contents

Presidential symposium

Advances in multiple sclerosis

1

Multiple sclerosis: how far away are we from Charcot?

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Jean-Marie Charcot (1825–1893) has the credit for the first clear description of the clinical manifestation of "la sclerose en plaques" or "la sclerose en plaques disseminées" and the correlation of the symptoms with the underlying pathology, through his widely read lectures [1–3].

In 1935, Rivers and Schwentker [4] introduced experimental allergic encephalomyelitis (EAE) in monkeys and the relevance of similar animal models has grown steadily. However, there is still no animal model that parallels MS exactly, although EAE has offered and still does much information regarding the mechanisms of demyelination and possible therapeutic interventions.

Charcot was also the first to delineate diagnostic criteria for the disease: the triad of nystagmus, intention tremor and scanning speech. However, Charcot did not propose the triad as formal criteria, but rather as characteristics of the disease. Later in 1954 the first true diagnostic scheme appeared by Allison and Millar [5] describing three categories: early, probable, and possible disseminated sclerosis. In addition, it was not until the 1950s that "multiple sclerosis" (MS) became the standard term in English [6], derived from the German term "multiple sklerose" and adopted in several languages terminology, thereafter.

Advances in diagnosis have occurred later, notably including the cerebrospinal fluid electrophoresis [7], evoked potentials [8–11], and magnetic resonance imaging (MRI) [12]. In order to determine disease activity and outcome (monitoring), several neurological scales for the rating of neurological impairment in MS have been proposed over the past 45 years; the disability status scale (DSS) and the expanded form (EDSS) were both introduced by Kurtzke [13, 14]. Several conceptual and practical problems with the EDSS limit its application as a precise neurological measurement and therefore, a single reliable scale for the clinical evaluation of disease progress is still not available. Poser's criteria [15] were for decades and still remain among the most widely used in MS diagnosis by the clinicians. However, it was inevitable that the rapidly growing importance of MRI in the diagnostic process would lead to recommendations for new diagnostic criteria for MS, which were developed by an international committee of neurologists and published in 2001; the so called McDonald criteria [16]. MRI findings are useful for the diagnosis of MS, they cannot be correlated to the clinical disability measured by EDSS, though. This finding raises questions about whether the so-called "MRI burden of the disease outcome" may have any clear value in the every day clinical practice. Nevertheless, Gd-enhancing lesions allow us to conclude about the disease activity no matter the clinical process. In addition, the occurrence of cognitive deficits in MS has recently been emphasized as an important clinical outcome. However, such an impairment has been recognized since 1877 when Charcot first observed "enfeeblement of memory" in his patients [17].

Recently, new insights regarding the timing and functional consequences of axonal loss in MS have attracted much attention to this issue after the findings of Trapp et al. in 1998 [18]. Previously McDonald et al. have suggested since 1992 that axon loss may be an important mechanism in MS [19]. However this knowledge is a renewed one, since Charcot also indicated the presence of axonal loss. Indeed, for several decades MS was considered by definition as a mainly demyelinating disease. Just recently it became clear that axonal injury might be the main contributor to the disability progress and other MS symptoms like cognitive impairment. However, the axonal loss and concomitant CNS atrophy are absent from the large multicenter clinical trials where all currently used therapeutic agents were tested. There is always a missing point since we proceed with studies performing several therapeutic interventions and still there are doubts about the value and the real meaning of both the clinical and laboratory outcome measures that are used. However, it is a common sense that all the therapeutic strategies currently performed with either immunomodulators or immunosuppressors are only partially effective.

Last but not least, the pathogenesis of the disease has been debated for decades and still is. Although the involvement of the immune system is well recognized, there are arguments that MS may not be an autoimmune disease [20].

Charcot's description of "la sclerose en plaques" was accepted to be so clear and accurate that Gowers stated 20 years later that little can be added to the description given by Charcot, and when Muller [21] wrote a book on MS in 1904, he referred to more than 1100 papers already published. Today, a century later, over 30,000 references are listed in PubMed under the term "multiple sclerosis". There is an increased amount of knowledge regarding the several immune pathways involved in the demyelinating and neurodegenerative process in MS, diagnostic procedures and treatment. However, the disease remains largely enigmatic in many aspects such as the pathogenesis i. e. autoimmunity vs. non-autoimmunity [20, 22, 23], the lack of an absolutely reliable animal model, the failure to control disability progress and neurodegeneration.

Hence, even today, Charcot's notion that "Disease is very old and nothing about it has changed. It is we who change as we learn to recognize what was formerly imperceptible", may at least partly be valuable. Presumably, if Charcot could be among us today he would enjoy the sophisticated techniques we use to identify and confirm his initial descriptions and skepticisms for the future in MS research and treatment. However, our current potential for a more effective diagnosis, monitoring and treatment allow us to be more optimistic than Charcot was.

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2
Neurodegeneration in multiple sclerosis
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Multiple sclerosis is an inflammatory demyelinating disease of the central nervous system, which implies that myelin sheaths are the prime target of the destructive process. It has, however, been noted already in the earliest pathological descriptions of this disease that axons and neurons are also affected. Axonal and neuronal injury in multiple sclerosis is important, since it is a major correlate of permanent functional deficit in the patients. Within demyelinated plaques the bulk of axonal injury occurs during the stage of active demyelination, but there is an additional slow burning axonal injury in chronic demyelinated lesions. Axonal destruction is mainly caused by products of activated macrophages and microglia, including oxygen and nitrogen intermediates or proteases, but there is an additional component mediated by excitotoxins. Demyelination and axonal loss within plaques can explain functional deficit in acute and relapsing MS, but this is the case only to a limited degree in secondary and primary progressive disease. In this stage of the disease profound damage also occurs within the normal appearing white matter (NAWM) as well as the cortex and the deep brain stem nuclei. Thus in progressive disease MS is no longer a focal disease of the white matter, but a global brain disease. Alterations in the NAWM and the cortex develop at least in part independently from those in white matter plaques. Global brain damage in progressive MS is associated with a mild, but diffuse inflammatory reaction, which appears to be compartmentalized behind a normal or repaired blood brain barrier. Tissue injury in the NAWM is mainly characterized by axonal injury and loss in the absence of primary demyelination. Although neurodegeneration in MS is in all stages of the disease associated with inflammation, the type of inflammation as well as the driving immunological mechanisms appear to be different and will require different treatment strategies.

3
Neuroprotection in multiple sclerosis
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Because persistent, non-remitting clinical deficits are in large part due to degeneration of axons within the brain and spinal cord, it is a high priority to develop therapies that will protect axons, so that they do not degenerate, in multiple sclerosis (MS). One approach (immunoprotection) is to target the immune attack, blunting it so that it does not result in neuronal loss. A parallel approach (neuroprotection) targets molecules within neurons so that, even in the face of immune attack, the injury cascade within them is blocked so that degeneration is halted. Axons have a different surface:volume ratio and express a different complement of molecules than neuronal cell bodies or synaptic components (presynaptic terminals/postsynaptic specializations) and thus are potentially amenable to different neuroprotective approaches. We have found that, after initial injury, a non-inactivating sodium conductance in axons permits a sustained sodium influx that drives the sodium-calcium exchanger (NCX) to operate in a reverse mode, importing damaging levels of Ca that lead to axonal degeneration. It is possible to almost fully protect axons in vitro so that they survive, and continue to function, after severe insults such as 60 minutes of anoxia, via exposure to sodium-channel blocking agents such as tetrodotoxin; clinically used sodium channel blockers such as phenytoin are also protective in vitro, although to only a partial degree. More recently, we have demonstrated that phenytoin is protective in vivo in an animal model of MS, experimental autoimmune encephalomyelitis (EAE) where it reduces the degree of axonal degeneration (loss of approx. 60 % of axons in dorsal corticospinal tract, untreated EAE; 20 %, phenytoin treated with serum levels within human therapeutic range), maintains ability to conduct action potentials in the preserved axons, and improves clinical outcome. In addition, we have demonstrated, both in EAE and in MS, that degenerating axons co-express the Nav1.6 sodium channel together with NCX; and have shown that the Nav1.6 sodium channel produces a relatively large non-inactivating sodium conductance. These results indicate that existing sodium channel blockers deserve further study as potential neuroprotective agents in MS, and suggest that development of Nav1.6-specific blockers may provide an even greater degree of protection.

4
Applying neurobiology to repair in multiple sclerosis
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Text not available.

Symposium

Imaging the human mind

5
The neural mechanisms of attention and its disorders
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Many patients who survive brain damage (following e. g. stroke, trauma) are left with cognitive deficits which adversely affect their functional recovery. Visuo-spatial neglect, typically resulting from right parietal cortex damage, and apraxia, typically resulting from left parietal cortex damage, are key deficits resulting from parietal cortex damage and pose intriguing neuroscientific issues and profound clinical problems. Both neglect and apraxia cannot be explained by either primary sensory or motor deficits, but rather an inability to pay the "appropriate" amount of attention to stimuli in contralesional space, or a disturbance of calling upon the relevant motor programmes and "time space engrammes" (Liepmann, 1905) of an action, respectively. From a clinical viewpoint these disorders hinder the patient's recovery and interfere with the ability to respond adequately to neurorehabilitation.

Lesion-based studies of action and visuo-spatial cognition have concentrated on such attentional/representational pathologies as spatial neglect and apraxia. Relevant theory has been characterized by such constructs as the neural simulation of action and guidance of motor behavior, or attentional vectors, local/global processing, and the visuo-spatial scratchpad. Such neuropsychology-derived models have taken us some way towards understanding a few basic principles of the "where" system and its connection and interaction with the action system. Recent functional neuroimaging studies have explicitly attempted to explore the underlying functional neuroanatomy implicated in traditional neglect and apraxia tasks and thereby allow a fairly direct comparison to be made with lesion-based results. These studies have highlighted the contribution of left and right posterior parietal cortex for object-related action and vision for action in space, respectively, and have stressed the functional significance of the polymodal areas along and in the human intraparietal sulcus.

6
Speech and language disorders: the contribution of neuroimaging
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The investigation of the neural mechanisms of speech and language has been based, until recently, on the so-called "experiments of nature", i. e. the study of the consequences of neurological disorders affecting the brain, typically stroke and dementia. The development of functional imaging techniques has tremendously increased the scope of this research field. Positron emission tomography and functional magnetic resonance, together with neurophysiological techniques, allow sophisticated studies of the spatio-temporal maps of language processing in the normal human brain. The results provided by these techniques have not only confirmed the basic tenets of clinical neuropsychology, but have also opened new bridges between the linguistic disciplines and clinical neurology. An example of this approach is the re-definition of the role of a classical language region, traditionally known as Broca's area. The results of imaging studies have shown its crucial role as an interface between motor processes and complex language functions, which include phonological processes and access to lexical-semantic and syntactic knowledge. Besides these and many other research implications, the new vistas on the language-brain relationship have clinical implications in several situations, such as the assessment of atypical dominance and the study of the neural correlates of aphasia recovery.

7
Hysteria and deception
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Text not available

8
From intention to action: the contributions of the frontomedian wall
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Imaging and patient studies have provided insights into the functional diversity of the frontomedian wall, but it remains an open issue whether and if so how this mosaic can be integrated into a more global model of frontomedian function. Two gradations can be suggested to underlie the functional organization of the frontomedian wall, one going radially from the cingulate (BA 24) and paracingulate cortex (BA 32) towards the cortical convexity, and hence from less granularized to full-blown six-layer cortices; the other one following a rostral-caudal direction from the pole frontal towards the supplementary motor area (SMA, BA 6) passing the median portions of BA 10, 9, and 8. In the present paper we propose that the latter gradation can be described as the way from intention to action. Here, the term "intention" refers to the presence and representation of a currently selected action goal (or set of goals), whereas "action" refers to the behavior that is meant to result in the achievement of the selected goal (or set of goals).

Ventral frontomedian areas BA 10m and 9m have been indicated in self-reflective thoughts and sensations (How am I? What do I feel?) including mentalizing or second-order intentionality ("theory of mind"). Patient data suggest that judgements of this kind may be bypassed by cognitive strategies without affective relation to the self. At the same time drive and volition, i. e., the expression of self-propelled will, can be extinguished in case of bilateral lesions in this compartment. Together, these findings give a hint that in the realm of action, BA 10m and 9m contribute to the building and maintaining of intentions and intentionality, i. e., beliefs, desires and related mental states.

Meta-analytic data suggest that particularly the ventral portion of this area, i. e. BA 10m, is associated with orbitofrontal structures which are known to provide affective context/meaning. The dorsal part in contrast, probably corresponding to BA 9m, receives input from a large cortical network including the temporo-parietal junction, and the precuneus. These regions among others are related to the representation of one's own body in relation to the environment, to the setting up of a situation model, and to mnemonic access to world knowledge, respectively. Together, BA 10m and 9m integrate affective and cognitive information needed for building of intention.

Adjacent BA 8m is known to be involved in decision making under uncertainty, i. e., action conflict. While decision making occurs on different levels on the way from intention to action, it here refers to the final step of selecting one out of several possible performances in order to achieve the selected goal. In contrast to ventrally adjacent anterior cingulate cortex (ACC) which is affected by response conflict and error detection, conflicts engaging BA 8m hence appear to arise whenever if-then rules of action are not available or those which are available do not guarantee successful performance. Functionally, BA 8m is hence a hybrid on the way from intention to action and marks a crucial transition point. It receives on the one hand teaching signals from dopaminergic areas in the midbrain and the ventral striatum calling for behavioral adjustments, and on the other hand from a variety of lateral cortical areas which provide information about the currently relevant information and environment.

Finally, data basis on BA 6 (pre-SMA and SMA proper) suggests this region to select and link precompiled motor subroutines stored in lateral premotor cortex. In contrast to the latter, this medial premotor system operates in action that is driven forward by prediction derived from an internal probabilistic model of the future. This latter notion integrates the final step of intention on its way to action, taking into account that the pre-SMA, in contrast to SMA proper, is not yet a motor area in the strict sense. Saying that pre-SMA provides an internal probabilistic model of the future means that this region serves the representation of the finally chosen to-be-produced action effects in a sequentially and hierarchically organized compound.

The way from intention to action is characterized by the consequent many-to-one reduction of options of both intentionality and behavior. In this context, two aspects deserve particular consideration. Firstly, the way from intention to action does not necessitate a step-by-step sequel of activation from BA 10 to BA 6. Processes may run in parallel, or iteratively back and forth. Secondly, and related to the first point, action is neither the only possible nor the obligatory outcome of computations of the frontomedian wall. Rather, intentions may continually come into one's mind without being finally translated into overt action. Likewise, the presence of some behavioral intentions, beliefs, and desires and their translation into action may be delayed and hence survive days, months and even years.

Cell and gene therapy

9
Cell therapy in Parkinson's disease
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Parkinson's disease (PD) is characterized by tremor, rigidity, and hypokinesia. Levodopa treatment is effective in the initial phase, but within 5 to 10 years, most patients develop fluctuating therapeutic responses and involuntary movements (dyskinesias). The main pathology underlying disease symptoms is a rather selective loss of dopamine (DA)-producing neurons in the substantia nigra leading to severe DA depletion in striatum. Implantation of DA-producing cells in denervated striatum seems to be a rational restorative approach to treat PD. The clinical trials in PD patients using intrastriatal transplantation of human fetal mesencephalic tissue, rich in post-mitotic DA neurons, have provided proof-of-principle that neuronal replacement can work in the human brain. However, it is unlikely that transplantation of human fetal mesencephalic tissue will become routine treatment for PD due to problems with tissue availability and too much variation in functional outcome.

For the development of an effective cell replacement therapy for PD, the work should now be focused on four major scientific issues:

(i) Generation of large numbers of DA neurons from stem cells. Stem cells could be useful as an unlimited source of specific neuron types, e. g., DA neurons. Hypothetically, DA neurons could be generated from stem cells of four different sources: embryonic stem cells from the fertilized egg, neural stem cells from the embryonic/fetal or adult brain, or from stem cells in other tissues. So far, neurons with characteristics of DA neurons have been generated from embryonic stem cells and from stem cells taken from the embryonic/fetal brain. However, the survival of these neurons after grafting has in many cases been poor and it is also unclear if they function as normal nigral DA neurons.

(ii) Improved patient selection. It is important to emphasize that just the availability of virtually unlimited numbers of DA neurons will not mean that we have a clinically competitive treatment for PD. We must define better criteria for patient selection with respect to stage and type of disease, and we must know the preoperative degeneration pattern so that we can define what should be repaired. Dopaminergic cell therapy will most likely be successful only in those patients who exhibit marked benefit in response to DA agonists, and in whom the main pathology is a rather selective degeneration of DA neurons. Debilitating symptoms in PD and related disorders are also caused by pathological changes in non-dopaminergic systems.

(iii) Improved functional efficacy of grafts. The transplantation procedure needs to be tailor-made with respect to the dose and location of grafted cells based on preoperative imaging so that the repair of the DA system will be as complete as possible in each patient's brain. It seems unlikely that DA neurons, predifferentiated from stem cells in vitro, will be able to induce a more pronounced improvement as compared to primary DA neurons in fetal grafts. Two major advantages with stem cells are, first, that the DA cells derived from stem cells can be standardized and generated in large numbers and, secondly, that they allow for controlled genetic modification which, hypothetically, could be used to increase, e. g., survival, migration, and function of their progeny. For a more complete reversal of PD symptoms, it may be necessary to develop tools to stimulate regrowth of axons from DA neuron grafts in the substantia nigra to the striatum, which probably will require modulation of host growth inhibitory mechanisms. It is currently unknown if immunosuppressive treatment would be needed in a clinical setting when the stem cells are of human origin.

(iv) Strategies to avoid adverse effects. New animal models are needed to reveal the pathophysiological mechanisms of graft-induced dyskinesias. The risk for teratoma from embryonic stem cells, and consequences of the introduction of new genes in stem cell-derived neurons should also be carefully evaluated.

10
Gene therapy in muscle diseases
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Patients suffering from inherited muscle disorders such as Duchenne muscular dystrophy are currently treated using supportive measures and palliative care improving both life quality and expectancy. However, curative treatment is not available and may be achieved only by specific molecular therapy. Various molecular approaches (such as gene therapy, stem cells, exon skipping) have been tested in vitro and in vivo, and the first clinical trials are underway. Adenoviral transfer of therapeutic genes such as dystrophin is hampered by low transduction efficiency of adult skeletal muscle. This is largely due to the lack of appropriate virus attachment receptors on the myofiber surface. Recent studies in transgenic mice revealed that upregulation of CAR (Coxsackie- and Adenovirus Receptor) improves gene transfer efficiency by approximately 10-fold. Conversely, the vector load that needed to be administered to achieve sufficient gene transfer could be lowered significantly. Reduced viral vector loads may help to control virally mediated toxicity and immunogenicity. Recent experiments have shown that directed mutagenesis of the adenoviral fiber knob allows for a significant reduction in CAR binding and for introduction of a new binding domain. Therefore, vector retargeting towards efficient and specific infection of skeletal muscle may be achieved by directed genetic alteration of adenoviral capsid proteins.

11
Gene, molecular and cellular therapy in ALS
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There is currently no cure for patients with amyotrophic lateral sclerosis (ALS). Riluzole, which has a small albeit consistent effect in clinical trials, remains the only therapeutic option. New treatment strategies based upon recently developed molecular and cellular tools are under study. Novel methods to deliver potentially beneficial substances to the nervous system and to motor neurons in particular, are being explored. This research concentrates on the motor neuron degeneration caused by mutations in the SOD1 gene, a reliable rodent model for human ALS.

Recent molecular developments allow the selective downregulation of the mutated SOD1, a strategy targeting the primary pathogenic event. The notion that cells surrounding the motor neurons in the ventral spinal cord may contribute to the degeneration of these neurons, has generated research into therapies that target microglial and astroglial cells. The replacement of degenerating motor neurons through stem cell technology has many biological caveats, although progress is being made. However, the finding that normalizing the cellular environment of motor neurons may attenuate their degeneration, opens perspectives for a different kind of stem cell therapy, which may be more realistic than pursuing replacement of motor neurons. Recent studies using viral delivery systems, but also using the direct administration of neurotropic factors into the nervous system, have suggested that the use of these substances remains an option in ALS, in spite of the disappointing results in the past.

All these approaches are in their early stages and much animal work remains to be done. Results obtained in mutant SOD1 overexpressing rodents need to be critically assessed for their significance to human ALS. Awaiting these results, clinicians have to develop methods to directly evaluate the neuroprotective rather than symptomatic effect of these treatments if applied to humans, and have to improve trial methodology. The development of diagnostic markers allowing the study of patients in very early or even presymptomatic stages of the disease will greatly facilitate the translation of animal data into human results.

However attractive some of these new approaches may be, basic and clinical researchers should make every effort to rigorously evaluate their effects before accepting them as an option for patients. It is the only way to fullfil the hope and meet the expectations of patients with this dreadful disease.

12
AdvHSV-tk gene therapy with intravenous ganciclovir increases survival in operable high-grade glioma
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High-grade glioma is a rapidly fatal brain tumour with no effective treatment. AdvHSV-tk, an adenoviral vector encoding the Herpes Simplex virus-thymidine kinase gene (EGO09, Cerepro), when used with subsequent ganciclovir may be a new effective therapy. Thirty-six patients (24 male, 12 female; age range 35–75 years) presenting at a single centre with operable primary or recurrent high-grade glioma between 1997 and 2002 were randomised to receive either AdvHSV-tk gene therapy (3 x 10^10 pfu) by local injections into the wound bed after the tumour was resected, followed by intravenous ganciclovir (5 mg/kg twice daily for 14 days) (n = 17) or tumour resection alone (n = 19). Patients in both groups with primary tumours received postoperative radiotherapy. AdvHSV-tk therapy increased mean survival by 81 %, from 39.0 ± 19.7 (SD) in control patients to 70.6 ± 52.9 weeks in patients in the active group (log-rank regression P = 0.0095). Median survival increased from 37.7 to 62.4 weeks, an increase of 65 %. The therapy was well tolerated as assessed by adverse events, clinical chemistry, haematology, and immunology. There was no evidence of any deterioration in quality of life or increased use of concomitant medications. AdvHSV-tk gene therapy with ganciclovir is a new, potentially effective therapy for operable high-grade glioma.

Controversies in neurology

13
Vascular versus degenerative dementia – is the distinction possible?
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The traditional dichotomy between degenerative (with insidious onset and gradual progression) and vascular dementia due to cerebral large vessel disease (with sudden onset and a stepwise course) has been fading when clinical and pathological studies have shown that small vessel disease – due most often to hypertension and diabetes – is associated with cognitive deterioration with insidious onset and gradual progression. Here, the most striking difference between degenerative and vascular etiology missing, the distinction between the two conditions is much more blurred and relies on soft signs and symptoms such as neuropsychological impairment profile (memory vs. executive functions), neurological exam (negative vs. mild bilateral pyramidal extrapyramidal and bulbar signs), gait (normal vs. slow and parkinsonian), and structural imaging findings (medial temporal atrophy vs. diffuse white matter lesions).

An additional variable that makes distinction difficult is the severity of cognitive impairment. In patients with well estabilished cognitive impairment – i. e. when the disability threshold has been exceeded – the physician can count on a large array of information coming from history (usually longer than 2–3 years), neuropsychology (tests where patients fail usually leave little doubt about normality), and behaviour, while in patients with mild cognitive impairment the available information is much more scanty.

A third variable is that the association of degenerative with small vessel vascular changes is the rule more than the exception in patients with cognitive impairment. The issue is thus: in a given patient with cognitive impairment, what is the relative contribution of degenerative vs. small vessel vascular changes?

These considerations account for the difficulty of an accurate estimate – to date – of the contribution of degenerative and vascular changes in patients with mild cognitive impairment and some degree of white matter damage. Some directions based on literature and anecdotal evidence will be provided.

14
Stroke prevention: how many treatments?
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Antithrombotic agents: For patients at high risk of arterial disease in general, allocation to antiplatelet therapy reduces the combined outcome of any serious vascular event (non-fatal stroke, non-fatal myocardial infarc-

tion, or vascular mortality) by about one quarter [1]. Aspirin is the most widely studied antiplatelet drug, with doses of 30–150 mg daily at least as effective as higher daily doses. For patients with transient ischaemic attacks or ischaemic stroke, however, the relative risk reduction is smaller: 13 % (95 % confidence interval 6–19 %) [2].

But is aspirin truly the best option? The alternatives are dipyridamole, clopidogrel, oral anticoagulants, or combinations of these drugs. For patients presenting with ischemic stroke, transient ischemic attacks, or any other form of arterial disease, dipyridamole is not more effective than aspirin [1, 3]. Clopidogrel has been compared with aspirin in a single trial (CAPRIE); it was marginally better than aspirin when the three different categories of arterial disease were analyzed together (relative risk reduction for major vascular events 8.7 % after almost 2 years; absolute risk reduction 0.51 %) [4]. Even if one disregards the disappointing fact that the result was not statistically significant for the stratum of more than 6000 patients with ischaemic stroke, still almost 200 patients would need to be treated with clopidogrel instead of aspirin to prevent a single vascular event. Walking an extra flight of stairs every day is probably more effective [5] – and cheaper. Oral anticoagulants do more harm than good in patients with cerebral ischemia of non-cardiac origin if the INR target value is 3–4.5 [6], are probably not more effective than aspirin with target values between 1.4–2.8 (median 1.90) [7], while their efficacy with intermediate intensities of anticoagulation is still under study [8].

The combination of dipyridamole and aspirin was superior to aspirin alone in a single, large trial [9], but not in four smaller trials performed earlier [1]. Other combinations of drugs are under study for patients with cerebral ischemia; their value is still unproven.

Antihypertensive agents: Another approach in patients with high risk of arterial disease is to lower the blood pressure, regardless of the baseline pressure. The so-called HOPE-trial confirmed the efficacy of this approach (22 % reduction of the composite outcome event) with the ACE-inhibitor ramipril (10 mg) in a broad range of patients at increased risk of cardiovascular disease [10]. In contrast, the PROGRESS trial concerned only patients (more than 6000) with cerebrovascular disease (transient ischaemic attacks or non-disabling stroke, including haemorrhages); the ACE-inhibitor perindopril, with or without the diuretic agent indapamide, reduced the risk of vascular events by 26 %, in those with 'hypertension' (> 160/90 mm Hg) as well as in those with lower blood pressures [11]. The key factor is probably the degree of blood pressure lowering rather than the type of drug used, because in a study of more than 33,000 patients with hypertension in combination with a second cardiovascular risk factor the efficacy of the thiazide diuretic chlorthalidone was at least similar to that of the calcium channel blocker amlodipine or the ACE-inhibitor lisinopril [12].

Lipid-lowering drugs: The Heart Protection Study studied the efficacy of 40 mg simvastatin against placebo in more than 20,000 patients with cardiovascular disease, diabetes mellitus, or hypertension; patients whose initial serum cholesterol level was below 3.5 mmol/l were excluded. The relative risk reduction of serious vascular events was 24 % in the entire group and 20 % in the subgroup of patients with cerebrovascular disease at entry [13, 14], regardless of the cholesterol level at entry. These results were largely confirmed in a placebo-controlled study of 40 mg pravastatin in 5804 elderly patients (70–82 years) at risk of vascular disease [15].

Conclusion: Patients with transient ischaemic attacks or non-disabling strokes (i. e. those still living independently) should – apart from specific contra-indications – currently be treated with

1. Aspirin (30–100 mg) per day.
2. One or more blood pressure lowering drugs, number and doses being dependent on the baseline blood pressure.
3. A statin, in a dose equivalent to 40 mg simvastatin.

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15
Management of optic neuritis

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Text not available

16
Prenatal diagnosis of adult onset neurological diseases

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Prenatal diagnosis in late onset neurological diseases is not a common request. This is due partly to the rarity of monogenic forms but also for the following reasons: a) the late onset implies that the expected child will live a normal life until the age of onset of the disease, usually in adulthood, b) prediction of the age at onset and severity are difficult especially on an individual basis, c) the absence of preventive treatment strategies makes predictive testing not a medical but a personal issue. The consequences are weighted differently according to the disease and the experience of the person at risk with the disease in the family. The variable clinical expression and reduced penetrance influence the decision in favour or against prenatal testing.

Huntington disease (HD) is the best studied late-onset autosomal dominant disorder for prenatal diagnosis. Interestingly only a minority of persons at risk for HD has chosen to prevent the transmission of the disease by the use of prenatal diagnosis. A larger number have undertaken presymptomatic testing.

In our 12 years experience with predictive testing in HD in France several observations have been made:

1) Less than 20 % of the persons at risk for HD will ask for presymptomatic testing and 40 % of those who want to know will finally not ask for the result.
2) The most frequent reason for testing is the need to know and not the reproductive decision making.

3) Pregnancies occurred in 28 % in non carriers compared to 15 % in carriers after presymptomatic testing (Evers-Kiebooms, 2002, Simpson et al. 2002).
4) The number of prenatal testing in carriers is rare (14 %) according to an European study (Evers-Kiebooms et al. 2002)
5) Ongoing pregnancy does not result in presymptomatic testing (Lesca et al. 2002).
6) Preimplantation diagnosis with indirect exclusion testing (without knowing the genetic status of the parent at risk) is an option taken by persons at risk (24 couples for two years in France, Moutou et al. 2004).

Similar results were obtained for other neurological diseases of late onset, such as autosomal dominant cerebellar ataxias with mutations in SCA1, 2, 3, 6, and 7 genes (Goizet et al. 2002). In myotonic dystrophy prenatal testing is more justified given the existence of the maternal inherited severe congenital form of the disease. For autosomal dominant inherited neuropathies or fascio-scapular-humeral myopathy, prenatal diagnosis requests are anecdotic. Presymptomatic testing and prenatal testing is an option which is not chosen by the majority of at risk persons. Adequate counselling before pregnancy and more importantly multidisciplinary accompanying of the at risk persons is essential.

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FREE COMMUNICATIONS
Oral Sessions

Session 1

Cerebrovascular disorders 1

O17

Decreased CSF hypocretin-1 (orexin-A) in patients after acute haemorrhagic brain stroke

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Background: The novel hypothalamic neuropeptides, orexins/hypocretins gained much attention, as they are potent modulators of different physiological functions. Acute brain injury due to haemorrhagic stroke is likely to affect posterior hypothalamus and its connections but its effect on the orexin system has not yet been studied. This study investigated the CSF hypocretin-1 levels in patients after haemorrhagic stroke (HS).

Methods: Nine patients admitted to the Surgical Intensive Care Unit after hemorrhagic stroke and 12 controls with other neurological disease (OND) were enrolled to this study. All patients had cisternal drains performed. Morning CSF samples were collected twice: first between day 1 and 2 after catheter insertion, and second sample between day 4 and 10 of the observation period. Patients were assessed on Glasgow Coma Scale (GCS) at disease onset and Glasgow Outcome Score in three months afterwards. Assay. Hypocretin-1 was measured blind to diagnosis by direct radioimmunoassay of 100 µL of CSF (Phoenix Pharmaceuticals, Belmont, CA; detection limit 40 pg/mL, intra-assay variation < 5 %).

Results: There was a significant difference in median CSF hypocretin-1 concentrations between OND controls (319.4 (302–361) pg/mL and HS patients (100.4 (0–139) pg/mL) ($p < 0.001$). Decreased concentration of hypocretin-1 in HS patients did not change over the observation period. There was no correlation between orexin-A levels and Glasgow Coma Scale (GCS) at disease onset or Glasgow Outcome Scale (GOS) at 3 months after disease onset.

Conclusion: Those results implicate the deficient hypocretin/orexin system to the complex pathophysiology of acute haemorrhagic injury to the brain.

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O18

Endovascular therapy of occipital arteriovenous malformations using ONYX™

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Objective: To describe the outcome of the combined treatment of occipital arteriovenous malformations (oAVM) using the liquid polymer ONYX and successive neuro-/radiosurgery.

Background: oAVM are congenital vascular anomalies which may cause visual symptoms, headaches, seizures, intracranial hemorrhage or progressive neurological deficits. Up to now, the preoperative embolisation of oAVM is mainly based on cyanoacrylate although it has several limitations (J Neurosurg 2003; 98:366–70). The potential advantages of ONYX result in precise and gentle neuro- and radiosurgical procedures (Clin Neuropathol 2002; 21:13–7).

Methods: 87 patients (pt.) with an intracranial AVM were treated with ONYX between 12/01 and 8/04. There were 14 oAVM in 13 pt. (8 M/5 F, 22–64 yrs.). 2 oAVM were asymptomatic, 7 were associated with headaches, 4 with seizures and 1 with an intracranial hemorrhage. 3 pt. had a visual field defect (VFD) before the embolisation: a quadranopia (QA) in 2 and a hemianopia (HA) in 1.

Results: 4 oAVM were occluded by embolisation alone. 9 were treated by embolisation and neurosurgery. Combined embolisation and radiosurgery is ongoing in 1. Mean occlusion rate after embolisation was 91 %. Angiography after surgery showed complete resection of the AVM in all cases. Clinical status after embolisation (n = 13) remained unchanged in 6 pt. and deteriorated in 7: a new minor VFD appeared in 1 pt., a new QA in 3, a new HA in 2 and an aggravation from QA to HA in 1. Clinical status after surgery (n = 9) was unchanged in 3 pt., improved from HA to QA in 1, and there was a deterioration in 5: a new QA in 2, an aggravation from QA to HA in 2 and a new HA in 1. VFD was without functional impairment in 4 pt. and could only be objectified by perimetry. Clinical and angiographic follow-up data were available in 9 pt. (average: 7.8 months, range: 3–16 months): There was a relapse of a small nidus in 1 pt. 8 oAVM remained totally occluded. 3 pt. remained without a clinical deficit (in one of them the QA resolved completely after 3 months), and there were 2 VFD without and 4 HA with functional impairment (3 of them had a VFD before the embolisation).

Conclusions: The application of ONYX allows endovascular monotherapy in almost a third of all pt. and a high nidal occlusion rate before neurosurgery. The benefit is a high and stable occlusion rate. However, the development or worsening of VFD either after embolisation or neurosurgery in the majority of pt. is therapy-immanent.

O19

Intima-medial thickness of the carotid artery and flow mediated dilatation of the brachial artery as indicators of endothelial dysfunction in acute stroke patients

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Background: Atherosclerosis is the pathological process underlying myocardial infarction, stroke and other occlusive vascular diseases. The development of techniques to accurately measure the early changes of atherosclerosis may aid in decreasing its morbidity and mortality. Objective: to assess endothelial function in ischaemic stroke patients by measuring the intima media thickness (IMT) of the carotid artery and the flow mediated

dilatation (FMD%) of the brachial artery using Duplex ultrasound techniques.

Subjects and methods: the study included 50 Egyptian patients (29 males and 21 females) presenting with acute ischaemic cerebrovascular stroke and 27 age and sex matched control subjects. Patients and controls were subjected to clinical evaluation, laboratory work-up, neuroimaging studies and Duplex ultrasound for the carotid and brachial arteries.

Results: Abnormal increase in the intima-medial thickness of the carotid artery was detected in 7 control subjects (25.9 %) and 33 patients (66 %) denoting endothelial dysfunction (ED). The FMD% of the brachial artery was abnormal in 7 control subjects (25.9 %) and 34 of the patient population (68 %) also denoting ED. A significant increase in IMT of the carotid artery and a significant decrease in FMD% and FMD-NTG of the brachial artery were found in stroke patients compared to controls ($p = 0.04$, 0.000, 0.01 respectively). The reduction of FMD% was significantly more as the number of risk factors for atherosclerosis increases, and a trend-wise increase in the IMT was observed with increase in the number of risk factors. Moreover, the IMT was significantly increased in the smoker patients than smoker controls ($p = 0.05$). Within the patient group, a statistically significant positive correlation was observed between IMT and the age of the patients and systolic blood pressure ($p = 0.000$, 0.05 respectively). No significant correlation was found between Duplex parameters with either initial severity or prognosis of stroke ($p > 0.05$).

Conclusion: FMD% of the brachial artery and IMT of the carotid artery are important markers for endothelial dysfunction and atherosclerosis in stroke patients. FMD% of the brachial artery is a valuable predictor of stroke occurrence.

O20

Psychiatric disturbances after acute subarachnoid haemorrhage

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Background and purpose: There are no systematic studies of the acute psychiatric symptoms in subarachnoid haemorrhage (SAH). This study investigates the presence and severity of psychiatric symptoms in acute SAH and their association with the amount and location of bleeding and hydrocephalus.

Patients and methods: We assessed 105 consecutive acute SAH (4 days) patients (mean age 54.5 years), using the Mini Mental State Examination (MMSE), Delirium Rating Scale (DRS), Denial of Illness Scale (DIS), Catastrophic Reaction Scale, Young Mania Rating Scale (YMRS), Apathy Scale (AS), Apathy Evaluation Scale (AES), Post Stroke Depression Rating Scale, and Montgomery Åsberg Depression Rating Scale. Patients with severe consciousness (GCS < 10) or language (NIHSS > 1) disturbance were assessed only with DRS. The amount and location of blood (total cisterns and fissures haematic densities; intraventricular haematic densities; pontine and superficial haematic densities) were measured on the admission CT-scan with the scale of Hijdra et al. Hydrocephalus was measured with the bicaudate index.

Results: Depression (58 %), apathy (31 %), cognitive impairment (27 %), denial (23 %), and delirium (13 %) were frequent in SAH patients. Higher amounts of total haematic densities ($p = 0.03$), intraventricular haematic densities ($p = 0.000$) and hydrocephalus ($p = 0.05$) were associated and correlated with lower scores in the MMSE. Higher DRS and DIS scores were associated and correlated with higher amount of intraventricular haematic densities ($p = 0.000$; $p = 0.002$) and with hydrocephalus ($p = 0.01$; $p = 0.000$). Higher AS and AES scores were correlated with higher intraventricular haematic densities ($p > 0.03$). Mania was present in two patients, one in the context of a delirium; both cases presented hydrocephaly and blood was equally distributed in both lateral ventricles. Higher YMRS scores were associated and correlated with higher total haematic densities ($p = 0.03$), intraventricular haematic densities ($p = 0.001$) and with hydrocephalus ($p = 0.04$).

Conclusion: Cognitive impairment, delirium, denial, and apathy were frequent in acute SAH patients. Cognitive impairment was associated with the amount of the subarachnoid haematic densities, with the amount of intraventricular haematic density and with hydrocephalus, while delirium, denial and apathy were associated with the amount of intraventricular haematic density and hydrocephalus.

O21

A functional MRI study of the motor system in patients with neuropsychiatric systemic lupus erythematosus

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Background: Several studies have demonstrated functional cortical changes in patients with several neurological conditions, including stroke, tumours and multiple sclerosis. The correlations found in some of these studies between the extent of fMRI activation and the extent and severity of brain damage suggest an adaptive role of these cortical changes.

Objective: To assess, using fMRI, the brain pattern of movement-associated cortical activations in patients with neuropsychiatric lupus erythematosus systemicus (NLES) and to investigate whether the extent of cortical reorganization is associated with the extent of brain pathology, measured on dual-echo images.

Patients and methods: We studied 14 right-handed patients with NLES (10 women and four men, mean age = 56.9 years, mean disease duration = 9.4 years) and 15 sex- and age-matched right-handed healthy volunteers. In each subject, we acquired: 1) fMRI during the performance of repetitive flexion-extension of the last four fingers of the dominant, clinically unaffected right hand, and 2) dual-echo turbo spin echo images. Lesion loads on dual-echo images were measured using a segmentation technique based on local thresholding. FMRI analysis was performed using SPM99 and a random effect analysis.

Results: Brain T2-visible abnormalities were detected in 11 NSLE patients (mean dual-echo lesion load = 3.1 ml, SD = 4.3 ml). Compared to healthy volunteers, NSLE patients showed more significant activations of the contralateral primary sensorimotor cortex (SMC), insula and cerebellum and the ipsilateral inferior frontal gyrus (IFG). They also had more significant activations of MT/V5 and the middle frontal gyrus, bilaterally. Dual-echo lesion load was significantly correlated with the activity in the contralateral primary SMC ($p < 0.001$, $r = 0.79$) and in the contralateral MT/V5 ($p < 0.001$, $r = 0.87$).

Conclusions: This study suggests that movement-associated functional cortical changes occur in patients with NLES and that they might be secondary to the extent of structural damage.

O22

A 3T diffusion tensor and voxel-based morphometry study of the brain of patients with migraine and T2-visible abnormalities

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Background: Functional MRI studies have shown changes in activation of several cortical and subcortical regions, including brainstem structures, in patients with migraine. However, structural MRI studies failed to show structural abnormalities in these regions.

Objective: To assess, using diffusion tensor (DT) MRI and voxel-based morphometry (VBM) the presence and extent of structural MRI abnormalities undetected using high-field MRI in patients with migraine with abnormalities on T2-weighted scans of the brain.

Patients and methods: We studied 16 patients with migraine (15 women and one man, mean age = 42.7 years, 7 with aura, 9 without aura) and 15 sex- and age-matched controls. Using a 3T scanner, we acquired: 1) brain T2-weighted images, 2) brain DT scan, with diffusion gradients applied in 32 noncollinear directions, and 3) high-resolution T1-weighted 3D magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence. Lesion loads on T2-weighted images were measured using a local thresholding segmentation technique. Mean diffusivity (MD) and fractional anisotropy (FA) histograms of the normal-appearing white matter (NAWM) and MD histograms of the normal-appearing gray matter (NAGM) were derived. An optimized version of VBM analysis was used to assess difference in GM volumes on MP-RAGE scans between patients and controls.

Results: In migraine patients, mean T2-weighted lesion load was 2.1 ml (SD = 2.9 ml). Compared to controls, migraine patients had increased MD histograms derived metrics of the NAGM ($p = 0.04$), while no abnormalities were detected in the NAWM. VBM analysis showed increased GM volume in the periacqueductal gray matter (PAG) in migraine patients compared with controls ($p < 0.0001$, corrected for multiple comparisons).

Conclusions: Structural abnormalities (possibly reflecting neuronal loss and gliosis) can be detected in the GM of patients with migraine using modern MR-based techniques. A more accurate assessment of the extent and location of these abnormalities might contribute to better understand the pathophysiology of the disease.

Session 2

Cerebrovascular disorders 2

O23
Thrombolytic therapy in pregnancy
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Thrombolytic therapy with tissue plasminogen activator (rt-PA) is an approved therapy for ischemic stroke, myocardial infarction, pulmonary embolism and thrombosis of cardiac valve endoprosthesis. In pregnant patients physicians are often reluctant to use thrombolytic therapy in fear of causing harm to the mother and fetus. So far 166 case reports using streptokinase and 22 case reports using rT-PA during pregnancy have been published. Rt-PA is now preferred over streptokinase due to its better safety profile and lower antigenicity. Indications for rt-PA thrombolysis were stroke (n = 8), thrombosis of cardiac valve endoprosthesis (n = 6), pulmonary embolism (n = 5), deep venous thrombosis (n = 2), and myocardial infarction (n = 1). One patient deceased (4.8 %) and three suffered from complications which were managed conservatively (13.6 %). This thrombolysis complication rate is similar compared to non-pregnant patients (death: 6 % for stroke, 2–3 % for myocardial infarction, and 6 % for pulmonary embolism; intracerebral bleeding: 11–20 % for stroke, 5 % for pulmonary embolism, and 1 % for myocardial infarction). Six out of the 22 fetuses died (29 %), but a likely causality to the prior thrombolysis could only be established in two (10 %) and is therefore comparable with the spontaneous rate of abortion. None of the live-born children suffered from a permanent deficit.

According to these data thrombolytic therapy should not be withheld in pregnant patients in case of life-threatening or potentially debilitating thrombembolic disease.

O24
Hypothyroidism and cerebral vein thrombosis – a possible association
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Introduction: There are many systemic illnesses that constitute risk factors for cerebral vein thrombosis (CVT). Thyrotoxicosis and hyperthyroidism have seldom been reported as a possible precipitating condition for CVT. Hypothyroidism was never associated with CVT. Nonetheless, there is growing evidence supporting a possible ethiopathogenic role of hypothyroidism in venous pathology. Several haemostatic and fibrinolytic parameters are disturbed in hypothyroidism. Hyperhomocysteinaemia, a known risk factor for premature venous thrombosis, has been documented in hypothyroidism. We report three cases of CVT in whom hypothyroidism was concomitantly diagnosed.

Clinical cases: Cases were women between 21 and 52 years old. They all presented with intracranial hypertension syndrome and symptomatic focal epilepsy. CVT was diagnosed with MRI and MR venography. Hypothyroidism was diagnosed in all patients in the acute phase of CVT. Two patients had autoimmune thyroiditis. None of the patients had diffuse goiter. Other risk factors for CVT were oral contraceptives in two patients and elevated homocysteine in one. All patients were treated with anticoagulation, thyroid replacement therapy and anti-epileptic drugs. All cases had a good clinical outcome, with normalization of thyroid function, remission of seizures and no recurrence of CVT at 1 to 3 years follow up.

Discussion: In the three cases reported here hypothyroidism was associated with CVT. Other risk factors for CVT were also present and the effect of chance cannot be ruled out. Further research is needed to determine a possible causal role of hypothyroidism on CVT and study direct interactions between thyroid hormones and venous haemostasis. We suggest that thyroid function should be included in the usual workup of CVT patients.

O25
In vivo detection of developing vessel occlusion in experimental focal cerebral ischaemia by iron particle enhanced MRI
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Background: Cerebral ischemia leads to necrotic tissue damage in the center of the developing lesion, but further extends into the periphery with time. Perfusion and diffusion weighted imaging indicate a potential mismatch between the infarct core and the surrounding tissue. In vivo assessment of intravascular thrombus formation may further improve the distinction between primary and secondary tissue damage.

Objectives: We report on a novel iron-contrast based MRI technique allowing assessment of ongoing vessel occlusion in experimental stroke.

Methods: Cerebral infarcts were induced in the rat by photothrombosis (PT). Animals concomitantly received superparamagnetic iron oxide (SPIO) particles giving rise to signal loss on T2-weighted MR images. The MR protocol included coronal T2-w and CISS sequences. A baseline MRI was performed immediately after infarct induction followed by a second scan at 1, 3, 24, 48 hrs or 14 d. Additional animals received SPIO particles with a delay of 2 and 24 hrs after infarct induction.

Results: Control animals exhibited hyperintense cortical lesions on T2-w/CISS images. Animals with concomitant SPIO application showed a hypointense lesion on MRI immediately after PT which corresponded to iron-laden thrombotic vessels within the lesion center. Three hrs later, this hypointense core was surrounded by a hyperintense rim. Vice versa, lesions exhibited a hypointense rim around a hyperintense core, when SPIO particles were applied 2 hrs after infarct induction. This latter finding indicates ongoing vessel occlusion exclusively at the periphery which became visible on MRI by intravascular trapping of SPIO particles. In accordance with our notion that we have visualized active periods of thrombotic vessel occlusion, delayed application of SPIO particles at 24 hrs after PT when thrombus formation had ceased already, showed merely hyperintense lesions, but no more hypointensities.

Conclusion: Systemically administered SPIO particles became caught intravasally during ongoing thrombosis of cortical vessels in focal cerebral ischemia and, thus, can serve as a MR marker for acute vessel occlusion in the brain. By comparing infarct areas showing hyperintensities (structural damage) and hypointensities (iron accumulation) on MRI, we could further show that in the PT model vessel occlusion is still ongoing despite cessation of illumination, and spreads from the core of the lesion to the "penumbra".

O26
Prevention of disabling and fatal strokes – acute outcome and long-term follow-up of carotid stenting in patients with asymptomatic carotid stenosis: a comparison of two different age groups
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Background: The 5-year-results of the ACST Trial have shown a significant benifit of immediate carotid endarterectomy (CEA) for patients with asymptomatic carotid stenoses aged < 75, but not for those > 75 years of age. We compared the acute and long-term results of carotid stenting of asymptomatic stenoses in these two age groups.

Patients: Results of carotid stenting of 233 asymptomatic stenoses in 223 patients younger than 75 years were compared to the results of 107 asymptomatic lesions in 104 patients older than 75 years. There were no other significant differences regarding baseline characteristics.

Methods: We used embolic protection devices in 85 % and 87.9 % respectively. 99.1 % and 98.1 % of the patients were treated with stent implantation. Follow-up investigations included a neurological examination and a carotid duplex scan before discharge, after 1 and 6 months. Thereafter whenever an event occurred. A questionnaire was sent every year.

Results: Concerning the patients younger than 75, the procedure was technically successful in all lesions. Within 30 days after the intervention 2 patients developed a minor stroke and 1 patient died due to non-cerebral cause. The stroke/death rate at 30 days was 1.3 %. Follow-up ranged from 1 month to 11 years (mean 1.1 ± 2.2 years, 433 pat. years). During follow-up the following events occurred: Ipsilateral major stroke in 2, ipsilateral minor stroke in 1, non-cerebrovascular death in 11 patients. Annual rate of ipsilateral stroke/cerebrovascular death was 0.7 %. In patients older than 74 years the procedure was technically successful in 102/104. Within 30 days of the procedure the following complications occurred: 1minor stroke, 3 major stroke and 2 non-cerebral deaths. The death/stroke rate at 30 days was 5.9 %. Follow-up ranged from 1 month to 7.5 years (mean 1.2 ± 1.7 years, 172 pat. years). During follow-up the following events occurred: Cerebrovascular death in 1, ipsilateral major stroke in 2, ipsilateral minor stroke in 1, non-cerebrovascular death in 13 patients. Annual rate of ipsilateral stroke/cerebrovascular death was 2.3 %. The differences between both groups were not significant.

Conclusion: Carotid stenting is a successful technique to prevent stroke and can be performed in patients older than 74. The peri-procedural risk seems to be higher in the elderly although the difference was not signifi-

cant. This has to be weighed up with the higher risk of surgery and natural course of the disease.

O27

Vascular pathology in 1011 first degree relatives of patients with intracranial aneurysms and in 812 first degree relatives of controls

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Background: Our aim was to compare by analysis of pedigrees the frequency of vascular pathology in first degree relatives (FDR) of patients with intracranial aneurysms (IA) and those in FDR of controls.

Methods: Pedigrees were made up for 194 patients with IA (94 men and 100 women) and in 193 age- and sex-matched control patients. On the first step all patients with IA and controls were interviewed about FDR: year of birth, disorders during their life, cause and year of death. As a second step we interviewed FDR with incomplete information and used all available medical documentation about FDR. As a third step we repeated the interview of patients with IA and their FDR 3 years after the initial episode. We included in the study only FDR with complete information about their disorders: 1011 FDR of patients with IA and 812 FDR of controls.

Results: We found that the frequency of strokes was 2.5 times higher in FDR of patients with IA than in FDR of controls: 6.5 % and 2.6 % respectively (RR = 2.52, 95 % CI = 1.56–4.09). Hemorrhages occurred 3.7 times more often in FDR of patients with IA than in FDR of controls: 3.4 % versus 0.9 % (RR = 3.90, 95 % CI = 1.74–8.75). Only 0.9 % of FDR of patients with IA had rupture of an intracranial aneurysm and nobody in FDR of controls. The frequency of headaches was 2.8 times greater in FDR of patients with IA than in FDR of controls: 19.6 % versus 7.1 % (RR = 2.74, 95 % CI = 2.08–3.62). FDR of patients with IA suffered from arterial hypertension two times more often than those in controls: 24.9 % and 11.6 % respectively (RR = 2.15, 95 % CI = 1.73–2.68). Coronary heart disease was 2.7 times more frequent in FDR of patients with IA than in those of controls: 8.9 % and 3.3 % (RR = 2.68, 95 % CI = 1.76–4.07). Strokes were the second main cause of death – after trauma – in FDR of patients with IA (11.9 % hemorrhages and 5.3 % ischemic strokes), whereas in FDR of control patients oncological disorders ranked second after trauma. Sudden death occurred in FDR of patients with IA 5.8 times more often than in FDR of controls: 11.1 % and 1.9 % respectively (RR = 5.68, 95 % CI = 1.37–23.39).

Conclusion: Vascular disease occurs relatively often in FDR of patients with IA. This confirms that vascular defects leading to the development of IA may be genetically determined, at least partly. We found that FDR of patients with IA were prone to the development of strokes, especially hemorrhages. Strokes represent the second main cause of death. Prevention of strokes and cardiovascular disease is necessary for FDR of patients with IA.

Session 3

Clinical neurophysiology 1

O28

Exaggerated inhibition of sympathetic nerve activity induced by arousal in phobic patients with a history of vasovagal syncope

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Arousal stimuli have recently been shown to elicit a reproducible transient inhibition of muscle sympathetic nerve activity (MSNA) in healthy subjects. Here we test whether this sympathetic response to arousal is altered in patients with a history of vasovagal syncope.

We studied 21 untreated vasovagal patients (30 ± 2 years; mean age ± SE): 10 with (32 ± 3) and 11 without (30 ± 3) blood/injury phobia. 17 healthy subjects (31 ± 2) were used as controls. MSNA was recorded from the peroneal nerve: heart rate (HR) and blood pressure (BP) were also monitored. Repeated trains of transcutaneous electrical stimuli (5 stim, 2 Hz), delivered randomly to a finger, were used to elicit arousal responses. Patients also underwent cardiological and neurological examinations, tilt test and a structured interview to investigate DSM-IV diagnostic criteria for specific phobia.

Vasovagal patients had significantly lower resting MSNA (28 ± 2 bursts/min) and diastolic BP (76 ± 2 mmHg) compared to controls (37 ± 2 bursts/min and 84 ± 3 mmHg; $p < 0.05$), whereas no significant differences were found for resting HR and systolic BP. The arousal stimuli induced prolonged reductions in MSNA and HR in phobic patients compared to non-phobic patients and controls. There was a small reduction of BP in phobic, but not in non-phobic patients (significant difference, $p < 0.001$).

Patients exhibiting vasovagal syncope had lower resting levels of sympathetic vasoconstrictor nerve activity. Furthermore, phobic vasovagal patients showed exaggerated inhibition of sympathetic nerve traffic (and bradycardia) in response to arousal stimuli. These neural characteristics might contribute to emotional syncope in stressful situations.

O29

Different patterns of trigeminal neuropathy in systemic sclerosis

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Introduction: Trigeminal neuropathy is one of the most frequent neurological complications of systemic sclerosis (SSc). However, its etiology is unknown. It is discussed whether apart from peripheral neuropathy there may also be central nervous system lesions. As brainstem reflexes can help to determine the site of the lesion, they were assessed in SSc patients presenting with sensory symptoms in the face, for better understanding the pathophysiology of the trigeminal involvement in SSc.

Patients and methods: Seven patients with diagnosis of SSc were included. Three of them also fulfilled criteria of secondary Sjögren's syndrome. Mean age was 53 years (range 43–63). Mean disease duration was 15 years (range 3–26). They all reported facial hypoesthesia and one patient also had facial pain. Symptoms involved V3 division in 5 patients, V2 division in 5 and V1 division in 4. They were bilateral in 3 patients. The following electrophysiologic variables were measured: R1, R2 and R2c response latency for blink reflex, jaw jerk latency and SP1 and SP2 latency for masseter inhibitory reflex.

Results: There were different patterns of involvement according to the results of brainstem reflexes.

Isolated or multifocal impairment of trigeminal nerve branches: two patients with absent silent period to unilateral mental nerve stimulation, and one patient with unilateral absence of R1 together with contralateral absence of jaw jerk.

Gasserian ganglia neuronopathy: two patients with absent silent period to mental nerve stimulation and delayed or absent R1 and R2 responses, with preserved jaw jerk.

Trigeminal nuclei dysfunction: two patients with unilaterally delayed or absent R2 responses but preserved R1 responses.

Conclusion: SSc patients with the same sensory symptoms in the distribution of the trigeminal nerve may have damage at different levels of the nervous system, including peripheral and central structures along the trigeminal pathway. The cause and mechanism of the damage may be different according to the site of the lesion. It is therefore advisable to complete these results with information from other studies (neuroimaging, immunologic and histopathologic findings) in order to further understand the pathophysiology of neurologic involvement in SSc.

O30

Bilateral corticospinal projections from the hand motor area in congenital mirror movements: neurophysiological evidence in a patient after stroke

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Objectives: To investigate functional correlates of congenital mirror movements. We describe a 76-year-old man with hereditary congenital mirror movements (MM) who had persistence of MM in the paretic hand during voluntary movements of the contralateral arm after an ischemic stroke.

Methods: The patient underwent clinical and MRI examination, somatosensory evoked potentials to median nerve stimulation, recording of movement-related potentials to self-paced voluntary movements of the left hand, and focal transcranial magnetic stimulation to both hand motor areas to record electromyographic responses to hand muscles of both sides.

Results: No movement was observed when patient attempted to voluntarily move the affected hand; no EMG responses were evoked to transcranial magnetic stimulation of the affected hemisphere. During performance of voluntary movements with the healthy hand or focal stimulation of the healthy motor area, mirror electromyographic responses of similar amplitude and latency were recorded. Mapping of short-latency somatosensory

evoked potentials only showed contralateral responses from stimulation of the healthy hand, while they were absent to stimulation of the affected side.

Conclusions: Our findings suggest the presence, at least in this patient with persistence of congenital mirror movement after stroke, of bilateral cortical projections to the spinal motor neurons. Whether this effect is mediated by the uncrossed portion of the pyramidal tract or by alternative mechanisms (e. g. bilateral projection of the crossed corticospinal tract at the spinal level) has yet to be clarified. Bilateral hand projections seem confined to the motor output or at least they do not seem to involve the lemniscal pathway.

O31
Generalised anhidrosis: different lesion sites demonstrated by microneurography and skin biopsy
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Generalized Anhidrosis (GA) shows a uniform clinical picture whether the pathogenesis involves lesion sites at several levels: hypothalamic thermoregulatory area failure, postganglionic sympathetic cholinergic nerve dysfunction, or intrinsic abnormalities of sweat glands. Laboratory studies are therefore needed to localise the specific lesion site.

We describe three patients who presented intolerance to heat and anhidrosis. The first patient showed GA at age 68 associated with postural hypotension. In the second patient symptoms started at age 33 years and were associated with absent tendon reflexes and a mydriatic right pupil unreactive to light. The last patient had been unable to sweat since birth.

Microneurography and morphological analysis of the skin and its innervation disclosed a different lesion site underlying GA in the three patients. The first patient showed a preganglionic autonomic nerve fiber lesion whereas the second patient presented a postganglionic autonomic nerve lesion. Sweat gland dysfunction was found in the third patient.

In conclusion, microneurography associated with morphological analysis of sweat glands and their innervation proved reliable diagnostic tools in detecting the site of the autonomic dysfunction responsible for GA in our patients, and in differentiating between a pre- or postganglionic autonomic nerve fiber lesion and sweat gland dysfunction.

O32
Subjective visual vertical modified by gravity
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Background: The task of judging whether a visual line is vertical or not requires integrating otolith and visual signals, and here we asked whether the known increased variance of adjustments of the subjective visual vertical (SVV) in different body tilt positions is caused by gravity- or retina-dependent factors.

Methods: 5 healthy subjects quickly (< 2 s) adjusted a luminous bar in darkness to either earth vertical or body vertical while in upright or tilted whole body positions. Paradigm A: 120 pseudo-randomized trials in upright, 75° right-ear-down (RED) or 75° left-ear-down (LED) position. Paradigm B: Subjects stayed in the same body position (upright or RED) for 40 trials, adjusting to earth and body vertical on alternate trials. Robust standard deviations (SDr: 10 % extreme data points discarded) and confidence intervals (CI) were determined.

Results: SDr of earth- and body-vertical adjustments in tilted conditions were not significantly different ($p > 0.01$). Paradigms A and B gave the same results, i. e. changing the whole-body position between trials was not an important factor. SDr in upright position was always significantly smaller than in tilted positions.

Discussion: Our results demonstrate that the increased variance in judgments of line orientation when subjects are tilted is not due to the orientation of the visual stimulus on the retina. Whether the higher variance in tilted whole-body positions is due to more noise from the otoliths, central processing limitations, or different strategies remains to be studied in both healthy subjects and patients with lesions along peripheral (utricular, saccular) or central gravity pathways.

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O33
Trigemino-hypoglossal silent period – A new pontomedullary brainstem reflex
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Introduction: Inhibitory connections between neurons of the sensory trigeminal nucleus and the hypoglossal nucleus have been described in the cat (Tomioka et al. 1999). We investigated if these inhibitory pontomedullary projections are also present in man by applying a newly developed stimulation and recording technique.

Methods: In a preliminary study we examined 14 healthy subjects (9 m, 5 f, age: 42 ± 13 years) with a specially designed enoral stimulation and recording device. The device allowed unilateral electrical stimulation of the trigeminal (V2) innervated palatal mucosa and simultaneous recording of the electromyographic activity from both halves of the tongue using surface electrodes. The stimulation intensity was at least 5-fold the perception threshold and was increased up to a maximum of 20 mA. During stimulation the subject pressed the tongue against the recording device. Five recordings were registered and the electromyographic activity was averaged and rectified.

Results: The examination was well tolerated by all subjects without adverse events. In all subjects we observed one bilateral suppression period of tongue muscle activity following unilateral electrical stimulation of the mucosal V2 afferents. The silent period started at 41.1 ± 4.7 ms and ended at 82.4 ± 12.5 ms. The mean duration of the silent period was 41.4 ± 10.2 ms.

Conclusion: According to animal studies, we demonstrated that inhibitory pontomedullary projections between sensory neurons of the enoral endings of the 2nd trigeminal nerve and hypoglossal neurons are also present in man. The silent period was bilaterally organized and showed in contrast to the exteroceptive suppression of masseter muscle activity only one supression period. The trigemino-hypoglossal silent period is most probably a nociceptive protection reflex and further investigations in patients with defined brainstem lesions have to clarify its diagnostic relevance.

Session 4

Clinical neurophysiology 2

O34
Ageing of the cortical ocular motor system on fMRI
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Neurophysiological studies have reported age-related effects in several parts of the ocular motor system, e. g., for upgaze or range and accuracy of vertical saccades [1], and of the vestibulo-oculomotor pathways, e. g., ocular motor responses to vestibular stimulation [2]. While the performance of horizontal optokinetic nystagmus (hOKN) remains relatively stable until the age of 50, it shows a small but significant decrease in more advanced ages [3]. The aim of this study was to determine whether these age-related changes are also present in the cortical ocular motor and visual systems during OKN measured by fMRI. This question is of special interest, since fMRI studies in patients of more advanced ages are increasing.

Thirty-three healthy volunteers (22 to 83y) divided into three equal subgroups (mean ages group I = 24.5, group II = 46.5, group III = 73.3y) were examined using a 1.5-T scanner (Siemens, Germany) and a T2*-weighted EPI sequence. Alternating blocks of hOKN (computer generated pattern of vertical black/white stripes) and the rest condition (stationary pattern) were applied. Subjects lay supine, their view being directed to a projection surface in front of the scanner bore. Eye movements were monitored online and recorded by a laser eye-tracking system (MReye-Track LR, SMI, Berlin, Boston). Random effects group and subtraction analyses were performed with SPM99b.

The groups I and II exhibited activations in a bilateral cortical network that was widespread in the visual cortex, including the lateral occipito-temporal cortex (MT/V5) and adjacent occipito-parietal areas, as well as in ocular motor structures such as the superior and inferior parts of the

frontal eye field, the prefrontal cortex, and the parietal eye field. Almost all these activations became less significant or were even absent in the older subgroup III. The contrast young (I) vs. old (III) revealed differences in the cortical eye fields and parts of the visual cortex. Furthermore, no specific areas were found only in the older subgroup or in the subtraction old minus young.

These findings of relevant reductions of the cortical activation pattern in the visual and ocular motor areas of older subjects are important for the interpretation of fMRI studies on brain plasticity or central compensation processes in patients of advanced age.

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O35

Multimodal (visual, motor, and somatosensory) evoked potentials as markers of clinical disability in relapsing-remitting and secondary progressive multiple sclerosis

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Background and Aim: Evoked potentials (EP) allow determination of functionally relevant demyelination in MS, generate numerical data potentially allowing a more powerful analysis of differences between single values than clinical scores. The aim of the present study was to determine the relation between multimodal EP and disability in MS patients at an early stage of the disease.

Methods: 16 patients with definite relapsing-remitting multiple sclerosis (RRMS) and 2 patients with beginning secondary progressive multiple sclerosis (SPMS) with relapses, diagnosed according to Poser or McDonald criteria and with a disease onset less than 10 years ago and Expanded Disability Status Scale (EDSS) score of 3.5 or less underwent combined visual evoked potentials (VEP), motor evoked potentials (MEP) to and somatosensory evoked potentials (SEP) from upper and lower extremities as well as a clinical neurological examination including determination of the EDSS-Score and 9-Hole Peg Testing (9HPT).

The Spearman rank correlation was used for statistical analysis.

Results: The sum of Z-transformed latencies of VEP, MEP and SEP to both upper and lower extremities correlated significantly with the EDSS ($rho = 0.55$, $p < 0.0202$), as well as the number of pathological results with the EDSS ($rho = 0.65$, $p < 0.0031$).

Conclusions: Multimodal EP yield numerical data that correlate well with clinical disability in patients with early RRMS and SPMS, even if the group is very small. Therefore, multimodal EP may be of possible use in future clinical trials in MS patients at an early stage of the disease, when modification of the disease course is most rewarding, and quantitative measurement most important.

O36

Saccade velocity in spinocerebellar ataxia type 2: a follow-up study

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Background: Spinocerebellar ataxia type 2 (SCA2) belongs to the autosomal dominant inherited polyglutamine diseases and is clinically characterized by progressive cerebellar ataxia, dysarthria, neuropathy and early slowing of horizontal saccadic eye movements. The underlying mutation is an unstable expansion of a polyglutamine domain within the protein ataxin-2. Slowed saccade velocity can be the presenting symptom even before ataxia manifestation. Since saccade velocity can be measured objectively and reliably we documented the horizontal maximal saccade velocity in Cuban SCA2 patients and its progression in a follow-up study after one year.

Methods: We analysed 82 Cuban SCA2 patients with polyglutamine repeat sizes ranging from 34 to 50 and disease durations from 1 to 42 years. The clinical status was assessed with the International Cooperative Ataxia Rating Scale. 80 healthy non-paid volunteers served as control group.

Within this cohort of 82 Cuban SCA2 patients we have the follow-up saccade velocity after one year determined in 46 patients at this time.

Horizontal saccades with amplitudes of 10°, 20°, 30° and 60° were recorded binocularly with silver-silver electrodes and a 2-channel Oto-screen AC electronystagmograph (Jaeger-Toennies, Höchberg, Germany) and analysed by MATLAB software and statistical tests.

Results: Maximum saccadic velocity (MSV) showed significant differences at 10°, 20°, 30° and 60° ($p < 0.0001$) between SCA2 patients and controls. Abnormal MSV was found already in patients with manifest ataxia for only 1 to 5 years. Stepwise linear regression analysis showed that 60° MSV was strongly influenced by polyglutamine size, and much less by disease duration.

After one year there was no significant decrease of MSV at 10°, 20°, 30° and 60°.

Conclusion: Saccade velocity is a sensitive, quite specific and objective disease marker which is strongly influenced by polyglutamine size and which might be useful to search for modifier genes. The saccade velocity is quite stable during one year of observation. Further follow up studies over 3 to 5 years are needed.

O37

Dissociation between detection and localisation of vibrotactile stimuli: a transcranial magnetic stimulation study

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Dissociation between detection and localization of tactile stimuli is a rare neurological phenomenon (Paillard et al. 1983). In the present study, we investigated in neurologically normal volunteers the potential role of SI in these two aspects of tactile perception. Participants (4F, 7M) underwent one experimental session of repetitive transcranial magnetic stimulation (rTMS: 900 pulses, 0.8 Hz, 90 % motor threshold) designed to induce a transient de-activation of SI. For each subject the TMS coil was positioned (using Brain Sight Frameless software) over the area of S1 previously identified in that subject (by fMRI) to be activated during a detection/localization task involving vibrotactile stimulation of the volar forearm. During sham sessions, conducted on separate days and counterbalanced for order, the TMS coil touched the target area on the skull, but was oriented away from SI. Detection and localization thresholds were assessed before and after rTMS or sham stimulation. The detection task required the subject to press a button each time he perceived a vibration on the volar surface of the right arm (21 one-sec stimuli of varying intensity and inter-stimulus interval); the localization task required the subject to identify, by pressing one of two buttons, the source of stimulation on each trial (one of two vibrotactile stimulators separated distal-proximally by ~ 2.5 cm on the volar surface of the right forearm; 1-sec stimulation, 30 % maximum stimulus intensity). Following rTMS – but not sham stimulation – we observed a 58 % increase in the vibrotactile threshold ($p < 0.05$), but no significant change in localization performance. These results are consistent with a dissociation of detection and localization processes. The differential effects of rTMS on the two tasks, and the lack of effect by sham stimulation, suggest a specificity of rTMS on reducing the detection of tactile stimulation through a transient de-activation of SI. The relative difficulty in interrupting performance of a localization task is in agreement with previous psychophysical studies (Harris et al., 2004). The potential involvement of SI in tactile localization deserves further study. Supported by the Canadian Institutes for Health Research.

O38

Multisensory vestibular cortex activated by otolith stimulation (fMRI)

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The multisensory vestibular cortical circuit on the basis of monkey experiments as well as human functional brain imaging studies includes the posterior insula, retroinsular areas, superior temporal gyrus, and inferior parietal lobule with the intraparietal sulcus. The aim of this fMRI study was to determine whether vestibular evoked myogenic potentials (VEMP) which have been a regular diagnostic instrument for sacculus function for the past years can activate the multisensory vestibular cortex in humans.

Therefore, the differential effects of unilateral VEMP stimulation (500 Hz tone burst signal in the right ear while the left ear was plugged for 26 sec, 13 runs) on cortical and subcortical activation were studied in 21 right-handed healthy volunteers (11 M, 10 F; mean age 24 years) lying supine in a clinical 1.5 T scanner wearing MRI-suitable piezo-electric headphones (Jaencke, Zuerich). The protocol included 172 volumes, each consisting of 40 slices of a T2*-weighted interleaved EPI sequence in alternating blocks of seven images at rest (eyes closed in the dark for the entire

trial), 6 during auditory stimulation. Each volunteer underwent three randomised sessions: 1. One with a 95 dB 500 Hz tone burst signal for which VEMPs had been recorded from the sternocleidomastoideus muscle outside the scanner before. 2. One session with a similar sub-threshold 65 dB 500 Hz tone burst signal which could not trigger VEMPs. 3. The third trial consisted of a 95 dB white noise signal. Random effects statistical analysis was done with SPM2 ($p < 0.001$ uncorrected).

In a paired t-test to exclude the auditory effects (95 dB click tone bursts vs. 65 dB click tone bursts) significant activations were found in a cortical network within both hemispheres but predominantly in the right hemisphere. This network included the middle frontal gyrus/sulcus (Brodmann area 10), the inferior frontal gyrus (BA 44), the superior and the medial temporal gyrus (BA 22, 29 and 42) bilaterally, the inferior parietal lobule with the supramarginal gyrus (BA 40), as well as the posterior insula.

This is the first demonstration by means of fMRI that monaural right-sided otolith stimulation in right-handers causes bilateral multisensory vestibular cortex activation with a dominance in the non-dominant right hemisphere similar to the activation pattern known from the stimulation of the entire vestibular nerve.

Session 5

Epilepsy 1

O39

Seizures on a neurologic intensive care unit

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Prolonged seizures are a common cause of hospitalisation. The underlying pathologies are various and rapid diagnosis is needed to adequately treat the patient. We retrospectively analyzed 653 consecutive patients admitted on a neurologic intensive care unit (ICU) during a two-years period. 127 patients (19.4 %) were admitted because of seizures. The underlying etiology was investigated by cerebral imaging (CCT and/or MRI) and in patients of no obvious pathology, by a cerebrospinal fluid analysis. The underlying etiology was: alcoholism 16 %, ischemic stroke 16 %, intracerebral hemorrhage 13 %, inflammatory diseases 12 %, metabolic disorders 10 %, brain tumours 8 %, microangiopathy 8 %, brain trauma 5 %, venous thrombosis 2.5 %, withdrawal of antiepileptic drugs in patients with known epilepsy 4 %, infection-associated 1.2 %. In 4.3 % no etiology was found. 37 % of all patients had a lumbar puncture, and 90 % of the patients with unknown etiology. Status epilepticus was found in 27.6 % (85 % convulsive, 15 % non-convulsive) of the patients. In all seizure-patients, EEG-findings varied from focal/lateral slowing (25 %), generalized slowing (40 %), epileptiform discharges (15 %) and PLEDS (10 %) to a normal EEG (10 %). According to the semiology, patients were treated with phenytoin (30 %), valproic acid (20 %), carbamazepine (16 %), a combination (29 %) or other AED (5 %). Thiopental anesthesia was conducted in 11.5 % of the patients suffering from status epilepticus. We conclude that prolonged seizures are a frequent cause of admission on a neurologic ICU with cerebrovascular diseases as the major underlying etiology.

O40

Zonisamide maintains or improves seizure control and is well tolerated over 2 years in patients with refractory partial epilepsy: interim analysis of an open-label extension study

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The long-term efficacy and safety of adjunctive zonisamide (ZNS), a broad spectrum antiepileptic drug (AED), was evaluated for up to 104 weeks in an ongoing open-label, multicentre extension study of patients with refractory partial epilepsy.

Patients aged ≥ 12 years (n = 243) completing an 18-week, double-blind, fixed-dose trial comparing placebo, ZNS 100, 300 or 500 mg/day were transitioned to ZNS 500 mg/day over 6 weeks prior to commencing a long-term, flexible dosing schedule. ZNS 100–600 mg/day, once or twice daily, and concomitant AEDs were adjusted in individual patients to optimise tolerability and seizure control. Efficacy was evaluated in terms of the median % change in frequency of seizures, relative to baseline data obtained at the final assessment of the double-blind trial, and the number of seizure-free days. Use of additional AEDs and incidence of adverse events (AEs) were also examined.

Efficacy was maintained or improved during long-term ZNS therapy, particularly in patients previously receiving 500 mg/day in the double-blind phase. Seizure frequency further decreased in patients treated previously with ZNS 500, 300 or 100 mg/day (complex partial (CP) seizure frequency −44 %, −13 % and −16 %, respectively; CP+ simple partial (SP) seizure frequency −33 %, −11 % and −28 %, respectively; all seizures (CP+SP+ all other seizures) −30 %, −6 % and −28 %, respectively) and in those receiving placebo during the double-blind phase (−31 % for CP, CP+SP and all seizures). Most subjects in the open-label phase continued or stabilised on a 500 mg/day dose. Overall, 130 (53.5 %) subjects were seizure-free for any full 28-day period, with 28 (11.5 %) seizure-free for 6 months, and 17 (7 %) seizure-free for 12 months. In this population of patients with refractory partial epilepsy, concomitant AEDs were reduced in 27 (11.1 %) patients, 4 (1.6 %) of whom converted to ZNS monotherapy. AEs were generally mild in severity and no unexpected events were reported. At interim cut-off 140 (57.6 %) patients had withdrawn; 28 (11.5 %) patients due to AEs and 44 (18.1 %) due to lack of therapeutic benefit. The incidence of serious AEs was low (35 patients; 14 %) and those leading to discontinuation (6 patients; 2 %) were considered unrelated to treatment.

These data demonstrate long-term efficacy and tolerability of adjunctive ZNS during flexible dosing.

O41

The effect of chronic levetiracetam administration on the development of spontaneous spike and wave discharges in genetic absence epilepsy rats from Strasbourg

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Purpose: Genetic Absence Epilepsy Rats from Strasbourg (GAERS) is a validated animal model of absence epilepsy. From PN30 on, age-related 'spike and wave discharges' (SWDs) start to appear on the EEG during spontaneous absence seizures. The aim of this study was to investigate the effect of early chronic levetiracetam (LEV) treatment on the development of SWDs in young and adult GAERS.

Methods: From postnatal day (PN) 23 until PN60, LEV (54 mg/kg, i. p.) was administered in GAERS (n = 8), while control GAERS (n = 7) received saline (0.9 % NaCl, i. p.). All animals were implanted with four epidural EEG electrodes at PN51. EEG was recorded for 3 h daily, during the last four days of the treatment (PN57-PN60) and during four additional days after treatment had been halted (PN61-PN64). The animals were monitored again at the age of four months, about two months after the last administration of LEV.

Results: During treatment (PN57-PN60), the mean epileptiform activity in the LEV group was significantly lower (62 %, $P < 0.05$) in comparison with the control group. During the following four days (PN61-PN64), the mean epileptiform activity was still lower (52 %) in the LEV group ($P = 0.064$), although treatment was already stopped. At the age of four months, there was no difference in epileptiform activity between controls and the LEV group.

Conclusion: In this study, chronic LEV administration induced a decrease in epileptiform activity in young GAERS. This effect persisted in some extent after treatment cessation (PN61-PN64), however at the age of four months all animals had developed similar spontaneous absence seizures.

O42

Definition of optimal parameters in electrical stimulation of the substantia nigra pars reticulata to suppress seizures in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS)

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Purpose: Pharmacological inhibition and high-frequency stimulation (HFS) of the substantia nigra pars reticulata (SNr) has been shown to suppress seizures in different models of epilepsy. The aim of the present study was to determine the most effective stimulation parameters in a genetic model in the rat (GAERS).

Methods: 46 males GAERS were stereotaxically implanted bilaterally with bipolar electrodes at the SNr and with monopolar electrodes on the cortex. The effects of acute 5-s unilateral and bilateral HFS of the SNr un-

der different stimulation parameters (polarity, phase, frequency and pulse width) were investigated on the occurrence of ongoing seizures. The optimal parameters for seizure interruption were then applied using different chronic stimulation protocols to obtain maximal seizure suppression and minimal effects of habituation (5 s ON 15 s OFF, 5 s ON 5 s OFF, continuous ON, demand stimulation, demand stimulation with a minimal intervall off 1 min). Upon completion of the experiments the results were analysed according to the localisation of the electrodes tips.

Results: Unilateral monopolar stimulation in SNr interrupted Spike and Wave Discharges (SWD) with a lower intensity than in Substantia nigra pars compacta (SNc) and surrounding areas. Within the SNr, significant lower antiepileptic thresholds were obtained at dorsal vs. ventral sites. The optimal parameters of acute SNr stimulation for SWD interruption were: bilateral, bipolar, monophasic, 60 Hz frequency and 60 µs pulse width. For chronic stimulations, only the demand protocol with a minimal off period of 1 min showed a significant decrease of seizure occurrence.

Conclusions: These results confirm that HFS stimulation of SNr can suppress absence seizures and show that (i) the dorsal part of SNr appears as the optimal location for HFS suppression of absence-seizures; (ii) bilateral, bipolar, monophasic, 60 Hz and 60 µs pulse stimulation are optimal parameters for acute stimulation; (iii) a minimal interval of 1 min is necessary to induce suppression of seizure upon chronic stimulation suggesting the existence of a desensitization phenomenon. Our data suggest the need of a closed loop stimulation procedure to chronically suppress seizures in therapeutical applications.

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O43

Efficacy of zonisamide in the treatment of refractory partial seizures in patients from an evaluable population

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The effectiveness of zonisamide (ZNS) in patients with refractory partial seizures was assessed in the evaluable population from a 36-week, double-blind, placebo-controlled trial. Patients had received ≥10 weeks of study medication and, based on the pharmacokinetic profile of ZNS, had reached steady-state drug concentrations. Use of this population allows an accurate assessment of ZNS efficacy.

Following a 12-week baseline period, 351 patients (aged ≥12 years; receiving 1–3 other antiepileptic drugs) were randomised (2:1:1:2) to placebo or ZNS 100, 300 or 500 mg/day. Doses were titrated over 6 weeks, which was followed by an 18-week, fixed-dose phase. Median percentage reduction in seizure frequency and responder rates (patients experiencing ≥50 % reduction in seizure frequency) for complex partial (CP), all partial (CP and simple partial) and all (CP, simple partial and secondarily generalised) seizures were assessed in the evaluable population. Safety was assessed through evaluation of adverse events (AEs) in all patients enrolled.

294 patients were included in the evaluable analysis. ZNS 500 mg/day produced significantly greater median percentage reductions in frequency of CP seizures (49.8 % vs. 16.3 %; $p < 0.001$) compared with placebo. Significant reductions compared with placebo were also observed in the frequency of all partial seizures, for ZNS 500 mg/day (48.9 % vs. 19 %; $p < 0.0001$) and 300 mg/day (44.7 % vs. 19 %; $p < 0.01$). Similarly, percentage reductions in all seizures were statistically greater than with placebo (18.7 %) for ZNS 500 mg/day (50.4 %; $p < 0.0001$) and 300 mg/day (40.9 %; $p < 0.01$). Responder rates with ZNS 500 mg/day were also statistically superior to placebo ($p < 0.001$) for CP (50 % vs. 20.9 %), all partial (48.8 % vs. 19.8 %) and all seizures (51.1 % vs. 18.3 %). A significant linear dose relationship was shown for responder rates for all seizures ($p < 0.0001$). Two patients achieved seizure freedom in both the placebo (1.8 %) and ZNS 500 mg/day (2.3 %) groups.

ZNS was well tolerated, with AEs generally of mild-moderate severity. The most common AEs were somnolence, headache, dizziness and nausea in the titration phase and headache and pharyngitis in the fixed-dose phase. The incidence of serious AEs was similar in ZNS- (up to 9.1 %) and placebo-treated patients (8.3 %).

These data establish ZNS as a well-tolerated and effective treatment for refractory partial epilepsy.

O44

Gamma knife radiosurgery – an alternative for intractable mesial temporal lobe epilepsy

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Introduction: The investigation of radiosurgery in the management of intractable Mesial Temporal Lobe Epilepsy (MTLE) is part of the quest of identification of novel therapeutic strategies for intractable epilepsy.

Methods: 5 patients with MTLE were treated with Gamma Knife (GK) after obtaining an informed consent. Age ranged from 23–44 years and the duration of seizures pre-GK ranged from 6–23 years with a seizure frequency of 1–4/wk.

MRI revealed right mesial temporal sclerosis (MTS) in 3, left MTS in 1 and bilateral MTS (left > right) in 1. Video-EEG and SPECT corroborated MRI findings. In the 5th patient, VEEG and SPECT localized to the left side which was therefore treated.

The target volumes of 6.9–7 cc encompassed the amygdala (sparing the supero-medial part), the head and anterior half of the hippocampal body and the anterior part of the parahippocampal gyrus. Two 18 mm collimators delivered a dose of 25 Gy to the 50 % isodose which corresponded to the target volume margin.

Results: 4 patients developed radiation reactions extending into the temporal lobe white matter at 8, 12, 13 & 14 months respectively. 1 patient lost to follow-up did not develop a reaction till 10 months when seizures had decreased marginally. All 4 who developed a radiation reaction experienced a dramatic decrease in seizures at the same time and went on to complete seizure remission. They have been followed for 2 years and all remained seizure and aura free except 1 patient who had 1 seizure on aggressive drug withdrawal.

Oral steroids were started at the time of appearance of the radiation reactions which were tapered depending on their resolution which took around 5–11 months. 1 patient developed dysphasia with mild right facial paresis which recovered following administration of methylprednisolone. 1 other patient was symptomatic for the radiation changes with diplopia which recovered. None developed raised intracranial tension, cognitive deficits or visual field defects or any steroid-induced complications.

Conclusions: Radiosurgery seems to offer the option of seizure control while sparing normal brain tissue and function unlike most surgical procedures but requires more investigation.

Though lower doses avoiding radiation reactions may be effective if followed for longer, the optimal dose for seizure control within a reasonable time-frame for MTS seems to be around 25 Gy to the target volume margin.

Session 6

Epilepsy 2

O45

Zonisamide improves seizure control in a dose-dependent manner in patients with refractory partial epilepsy

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The dose relationship to efficacy of zonisamide (ZNS) was investigated by assessing changes in seizure frequency during adjunctive treatment of patients with refractory partial epilepsy.

Patients aged ≥12 years (n = 351) with refractory partial seizures (receiving 1–3 other antiepileptic drugs) were randomised to placebo, ZNS 100, 300 or 500 mg/day (2:1:1:2) in a double-blind study. An upward dose-titration period (6 weeks) was followed by an 18-week, fixed-dose assessment phase.

Efficacy endpoints were evaluated for each treatment group in 312 patients with partial seizures without secondary generalisation (modified ITT population: patients with partial seizure frequency data from the fixed-dose phase). These included: responder rates (≥50 % reduction in seizure frequency), seizure freedom rates, exacerbation rates (> 25 % increase in seizure frequency), and assessment of the percentage reduction in seizure frequency.

The proportion of responders and patients who became seizure-free increased in relation to ZNS dose. Responder rates were 20.2 %, 28.8 %,

42.9 % and 50.5 % ($p < 0.001$ vs. placebo) with placebo, ZNS 100, 300 and 500 mg/day, respectively. Corresponding seizure freedom rates were 2.8 %, 0 %, 4.8 % and 9.1 %. Conversely, seizure exacerbation rates decreased with increasing ZNS dose, with rates of 13.8 %, 13.5 %, 4.8 % and 4 % reported with placebo, ZNS 100, 300 and 500 mg/day, respectively. Analysis of seizure frequency data shows a similar dose-response relationship. The majority of patients in the ZNS 500 mg/day group exhibited a > 50–75 % (26 % patients) or > 75–100 % (24 % patients) decrease in seizure frequency. The majority of those receiving 300 mg/day experienced a > 25–50 % decrease (33 % patients). A > 0–25 % decrease was observed most frequently in patients receiving 100 mg/day (29 % patients) and placebo (30 % patients). Similar dose-dependent changes were observed for complex partial and all seizures.

These data indicate that ZNS reduces seizure frequency and seizure exacerbation in a dose-dependent manner in patients with refractory partial epilepsy, with the greatest efficacy observed with the ZNS 500 mg/day dose.

O46

The protective effects of levetiracetam on mitochondrial function after status epilepticus

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Mitochondrial dysfunction occurs as a consequence of prolonged epileptic seizure activity, and has a role in the associated brain damage (Cock et al. Ep.Res. 2002). Agents that prevent mitochondrial damage may prevent neuronal death and dysfunction. Levetiracetam (LEV) is a novel antiepileptic drug, with a unique mechanism of action (Lynch et al. PNAS 2004). Using the perforant pathway stimulation model of status epilepticus (SE), we measured hippocampal mitochondrial enzyme activity and reduced glutathione (GSH) levels post SE, and determined the effects of LEV.

Stimulating and recording electrodes were implanted into the right perforant pathway and dentate granule cell layer, respectively, of anaesthetised rats. 6 days later, SE rats underwent 2h of stimulation (2–4 mA, 20Hz) following which self-sustaining chronic limbic status ensued. EEG and behaviour were recorded throughout. Intraperitoneal (ip) LEV or saline (controls) was given at the end of stimulation. Diazepam (20 mg/kg, ip) was administered to all animals to terminate seizures after a further 3h. Rats were sacrificed 44h later and the hippocampi dissected into CA1, CA3 and dentate gyrus/hilus (DG/H). Mitochondrial enzyme activities (spectrophotometric assays) and GSH levels (electrochemical HPLC) were determined in shams ($n = 16$) and SE rats given saline ($n = 10$); 200 mg/kg LEV ($n = 5$); or 1000 mg/kg LEV ($n = 5$).

Although LEV administration did not abolish SE, rats that received 1000 mg/kg exhibited reduced spike frequencies in their EEGs. Consistent with our previous studies, significant biochemical changes following SE (saline) rats when compared to shams (ANOVA and Tukey, all $p < 0.005$): alpha-ketaglutarate dehydrogenase activity was reduced in all hippocampal regions (CA1: –18 %; CA3: –24 %; DG/H: –18 %), as was aconitase activity (CA1: –25 %; CA3–34 %; DG/H –27 %). Complex I activity decreased in CA3 (–15 %); citrate synthase activity fell in CA1 (–8 %) and CA3 (–8 %). GSH levels in CA3 were also significantly attenuated post SE (–25 %); 200 mg/kg LEV conferred no significant protection, but in rats that received 1000 mg/kg LEV no mitochondrial dysfunction/GSH decline was present.

We confirm that SE results in reduced activity of essential mitochondrial enzymes, known to be sensitive to free radical damage. Administration of high dose LEV (1000 mg/kg) protects against this mitochondrial dysfunction, indicating that in addition to its antiepileptic actions, LEV may have neuroprotective effects.

O47

Predictors of death in the emergency room in 107 patients with EEG-confirmed status epilepticus

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Status epilepticus (SE) is a neurological emergency with high mortality. Whereas an aggressive therapeutic approach appears justified for generalized convulsive and subtle SE, there is no consensus for less severe presentations. Identifying early factors predictive of outcome could help minimize the risk of over-treatment. Previous studies showed that age and acute symptomatic etiology are related to death; other variables (time to treatment, gender and ethnicity) are controversial. Level of consciousness has received relatively little attention.

Methods: We screened the discharge database (1997–2004) of two tertiary care hospitals for patients with EEG confirmed SE. Subjects with anoxia, insufficient data, or age < 16 were excluded. Seizure semiology and level of consciousness (alert, somnolence, stupor, coma) were assessed according to the first available description. Etiologies were labeled as "PFE" if potentially fatal independently of SE. SE was classified as subtle, generalized convulsive, complex partial, or simple partial. We performed uni- and multivariate analysis.

Results: Of 240 patients, we excluded 133 (13 anoxia-ischemia, 87 insufficient data, 33 children), and included 107, accounting for 127 episodes: 50 males, median age 52 (16 to 97), 79 Caucasians. Mortality was 15.6 % among 96 patients with a 1st SE episode; 10 of them also experienced recurrent SE (rSE). 11 patients only had rSE. Among these 21 patients with rSE, mortality was 4.8 %. For the 1st SE episode, after multiple logistic regression, death was associated with age 65 or greater ($p = 0.02$), PFE ($p = 0.01$), and deep consciousness impairment before treatment ($p = 0.04$), but not gender, history of epilepsy, SE type, or time to treatment. Caucasian ethnicity was only predictive in univariate analysis.

Conclusion: At initial evaluation, older age and severity of consciousness impairment are predictive of death in SE. Since etiology, although strongly predictive, is rarely identifiable at the beginning of treatment, these features may help clinicians to orient early their therapeutic strategy. Surviving a 1st SE episode appears to lower the mortality of subsequent episodes, a new observation supporting the idea that clinical background, rather than SE per se, determine mortality. A prospective study is needed to clarify how aggressively patients with mild consciousness impairment should be treated (Supported by the Swiss National Science Foundation and the SICPA Foundation).

O48

Focal delivery of standard antiepileptic drugs in the tetanus toxin model of epilepsy in rats

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Purpose: To compare the efficacy and tolerability of systemic versus focal (site-specific) drug delivery in a model of refractory cortical epilepsy in rats.

Methods: Chronic focal epilepsy was induced by injecting 50 ng of tetanus toxin or vehicle alone (controls) into the motor neocortex of rats as previously described (Nilsen, Epilepsia 2005). Antiepileptic drugs were administered systemically (intraperitoneal, phenytoin 50 mg/kg, diazepam 10 mg/kg) injection, or directly to the seizure focus (Phenytoin, Tiagabine) using a pre-implanted cannula. Seizures were monitored prior to and following single applications, using EEG, facial EMG and behavioural observations, again as previously described. Data were analysed at a later date, with the researcher blinded to the treatment group. Statistical analysis was undertaken using ANOVA.

Results: Tetanus toxin induced frequent and mild behavioural seizures with EEG and EMG correlates in all animals as previously described. In keeping with our previous preliminary studies of drug responsiveness, seizures were refractory to systemic administration of diazepam and phenytoin at doses causing significant sedation. (E. g. % seizure time pre-Phenytoin 43.6 ± 7.26 % pre-injection vs. 45.3 ± 6.14 % post-injection; not significant). Focal delivery of phenytoin ($N = 10$) to the seizure site reversibly reduced EEG and behavioural seizure activity. Focal delivery of tiagabine ($N = 4$) or saline ($n = 7$) had no significant effect on the seizure profile. There were no detectable side effects from either drug administered focally, or in the control group.

Conclusions: In keeping with our previous studies, we have further demonstrated that this model is refractory to high dose systemic antiepileptic drugs, including at systemic doses causing marked sedation. This is thus an ideal model for assessing novel treatments for refractory epilepsy. Despite lack of efficacy at high systemic doses, delivery of phenytoin directly to the seizure focus significantly reduced seizure activity without observable side effects. Site specific drug delivery offers a novel treatment approach for refractory cortical epilepsy, and long-term in vivo studies are required to investigate this further.

O49

Surgery for intractable post-traumatic epilepsy

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Introduction: Despite early beginnings by Penfield and Foerster in the surgical treatment of intractable post-traumatic epilepsy (PTE) caused by en-

cephalomalacias, there are very few studies detailing the results of surgery for this entity which is what we sought to achieve here.

Methods: We did a retrospective review of 9 patients who had undergone resections of encephalomalacias as treatment of intractable PTE.

Presurgical evaluation included EEG/video-EEG/SPECT and a MRI employing FLAIR sequences.

Focus excision was done using intraoperative electrocorticography.

Results: The mean interval between trauma and the onset of intractable epilepsy was 4 years. The median duration of suffering from epilepsy before surgery was 8 years (3–29 years), reflecting a tendency to persist with medical treatment, probably because surgical treatment for intractable PTE is not very popular a notion. The median seizure frequency was 10/month.

Frontal encephalomalacia was most common with 6 patients having unilateral-frontal foci on MR, 1 having a parietal focus and 2 having bilateral-frontal foci.

Among 7 patients with a single focus on MRI, VEEG revealed discordant foci in 2 and EEG revealed discordant foci in 4. All patients with discordant foci on VEEG/EEG ultimately underwent resection of the MR-identified-focus with Class-I seizure-outcome. Therefore with clear-cut MRI-localization, EEG/VEEG has mainly adjunctive value and discordance with MRI should not provoke fears. SPECT/VEEG/EEG played a greater role in the 2 patients with bilateral-frontal foci on MRI. Both had more MR changes on the right which was confirmed to be the seizure focus with VEEG/SPECT.

All 9 patients had Engel's-Class- I seizure-outcome at a mean follow-up period of 24 months (12–63 months).

Intra-operative ECoG was helpful in deciding the extent of resection in that all patients had no post-op spikes and had Class-I outcome.

Functional-MRI had been done in 2 patients and awake-craniotomy in 1 in view of close proximity of eloquent cortex to the surgical focus. Only 1 patient with a parietal focus had transient hemiparesis postoperatively.

Conclusions: The excellent results demonstrated following encephalomalacia resections for intractable PTE should prompt search with a MRI using FLAIR sequences for resectable foci among these patients. Surgical treatment should not be delayed. Intraoperative ECoG helps in determining the extent of resection required.

Session 7

Motor neuron disease 1

O50

Transcriptional regulation of inflammatory processes in amyotrophic lateral sclerosis: expression of CCAAT/enhancer binding protein (C/EBP) beta and suppressor of cytokine signalling (SOCS) 1

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Elevations of numerous proinflammatory cytokines and chemokines in amyotrophic lateral sclerosis (ALS) tissue indicate that inflammatory mechanisms may contribute to motor neuron death in this disease.

However, little is known about exact functions, interactions, and the regulation of these molecules in ALS pathogenesis.

We used gene expression profiling and immunohistochemical techniques to identify an elevation of CCAAT/enhancer-binding protein-β (C/EBP β) as well as suppressor of cytokine signalling (SOCS) 1, which may act as regulators of inflammatory signalling in ALS.

CCAAT/enhancer-binding protein-β (C/EBP β) is a transcription factor that belongs to the basic leucine zipper class. It couples extracellular signals to various intracellular processes like growth and differentiation but also participates in various inflammatory processes. The suppressor of cytokine signalling (SOCS) family comprises proteins induced on cytokine stimulation, which further block signalling in a negative feedback loop. Within this family, SOCS1 is the most potent inhibitor of cytokine signalling.

Microarray analysis revealed enhanced expression of C/EBP β and SOCS1 in the spinal cord of ALS-patients as well as in SOD-transgenic mice, an animal model of familial ALS. Furthermore, several transcriptional targets of C/EBP β, including interleukine 6 (IL-6) were up-regulated in the same specimens. Immunohistochemistry showed that C/EBP β and SOCS1 were mainly expressed in microglial and astroglial cells of the spinal cord ventral horn.

Since IL-6 is not only a transcriptional target, but also a strong inductor of C/EBP β expression, elevated levels of these molecules may be part of a feed-forward mechanism causing the spreading of the inflammation. Conversely, expression of SOCS1 may be an attempt to limit inflammatory processes in ALS.

O51

Oxidant-generating and antioxidant systems in cerebrospinal fluid in amyotrophic lateral sclerosis

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Etiopathogenesis of Amyotrophic Lateral Sclerosis (ALS), a progressive disorder with upper and lower motorneurone degeneration, is unknown. However, accumulating evidences, including the occurrence of mutations in superoxide dismutase 1 (SOD1) gene in 20 % of familial cases, indicate that oxidative stress is involved in the pathogenesis of this disorder, likely due to an imbalance between antioxidant and oxidant-generating systems within the affected cells. To this respect proteins in cerebrospinal fluid (CSF), owing to their high rate of spontaneous oxidation, can represent a useful marker for oxidant status, also in ALS. Aim of this study has been to assess oxidative stress in ALS patients by combining spectrophotometric detection of the advanced oxidation protein products (AOPP), ferric reducing ability (FRA) and ELISA assay of nitrite/nitrate ratio, a relative index of nitric oxide (NO) production, in CSF.

49 patients and, for comparison, 8 sex-matched controls with different neurological disorders but normal standard CSF examination were included in the study. The patients with ALS were divided into groups according to age (patients with ≤ 60 years and with > 60 years), symptoms (bulbar, peripheral and mixed) and presence or not of SOD1 gene mutations. AOPP (mmol/l) resulted higher in patients with ALS than in controls (mean ± SE: 13.2 ± 1.7 in patients with ALS and 1.7 ± 1.0 in controls), but within patients there was a significant difference between bulbar and peripheral form (0.0 vs. 15.5 ± 2.0, respectively); AOPP mean value in mixed form was intermediate (10.5 ± 3.2). The analysis of global variance was for $p = 0.0024$. In patients as well as in controls AOPP values were not influenced by age. FRA (mmol/l) was lower ($p < 0.03$) in whole patient sample (48.8 ± 2.2) compared to controls (61.3 ± 3.2), and, marginally ($p = 0.05$), in bulbar (45.2 ± 2.4) with respect to the other forms (55.8 ± 6.0). There were no differences in AOPP and FRA values in patients with Familial ALS ($n = 4$). Although the total amount of nitrite + nitrate levels was similar in CSF from patients and controls, the nitrite proportion was 60 % of total in ALS subjects, 35 % in controls.

These data show that oxidative stress occurs in cerebrospinal fluid in ALS patients. Furthermore, based on the observed differences between subgroups of patients, it might be hypothesised that oxidative stress is differently involved in the pathogenesis of peripheral and bulbar forms of ALS.

O52

Low incidence of Type 1 Spinal Muscular Atrophy in a Caribbean population of mixed ethnicity

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Type I Spinal Muscular atrophy is one of the most common autosomal recessive diseases of childhood, with an estimated incidence of 1 in 10,000 live births and a reported carrier frequency of 1 in 50. There have been few population-based studies of SMA.

Our objective was to study the incidence and prevalence of Type I Spinal Muscular Atrophy in Cuba from 1996–2002, taking into account the colour of the skin of the patients as a surrogate marker of ethnicity.

A database of all cases of SMA in Cubais retained at the National Institute Centre for Neurology and Neurosurgery in Havana.

We conducted a retrospective analysis of all cases of Type I SMA over a 6 year period.

The overall incidence of SMA 1 in Cuba was 3.57/100,000 live births. 84 % of cases had deletions in exons 7 and 8 of the SMN1 gene. When the population was segregated by skin colour, the incidence was 8/100.000 for whites; 0.89/100,000 for blacks, and 0.96/100,000 for those of mixed ethnicity.

Our findings suggest that Type I SMA may occur less frequently in those of African ancestry.

O53

Lack of association of the vascular endothelial growth factor haplotypes and risk for ALS in Irish and North American populations

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In an experimental model, transgenic mice with a specific deletion of the hypoxic response element of the vascular endothelial promoter resulted in an ALS phenotype. In a meta-analysis of over 900 ALS patients and more than 1000 controls from Northern Europe, individuals homozygous for the VEGF haplotypes –2578A/–1154A/–634G/ or –2578A/–1154G/–634G/ have a 1.8 times greater risk of developing ALS. These at-risk haplotypes were associated with reduced VEGF expression and lowered serum VEGF levels.

We sought to examine these at-risk haplotypes in the Irish and North American populations. We examined the VEGF haplotypes in a total of 466 sporadic ALS (SALS), 77 familial ALS (FALS) (no SOD1 mutations) and 408 matched control samples.

Analysis of the VEGF haplotype in the Irish population showed no significant difference between SALS individuals and controls ($p > 0.36$). Analysis of the North American population identified an increase in allele frequency of the –2578C, –1154G and –634C alleles in SALS and FALS when compared to controls ($p > 0.024$). However there was no significant difference in the distribution of the at-risk VEGF haplotypes in SALS/FALS when compared to controls ($p > 0.05$). Furthermore combined analysis of the North American and Irish populations showed no significant difference in the at-risk VEGF haplotype when SALS were compared to controls ($p > 0.3$).

There is increasing evidence strongly suggesting a biological role for VEGF as a modifier of motor neuron degeneration. However, this study does not replicate the haplotype association identified in a large cohort of Northern Europeans.

O54

Cognitive impairment and regional cortical atrophy in amyotrophic lateral sclerosis

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Background: Patients with amyotrophic lateral sclerosis (ASL) show a cognitive impairment consistent with frontal-temporal dysfunction. In spite of histopathological evidence of cortical atrophy in parietal-frontal-temporal areas, data on brain MRI volume measures are discordant.

Objective: To correlate cognitive profile with regional cortical volumes measured by voxel based morphometry (VBM)in ALS.

Patients and Methods: Forteen clinically probable or definite ALS nondemented patients (El Escorial criteria) were examined. Age at observation was 57.85 ± 10.24 years and disease duration was 53.6 ± 35.5 months. Functional status was assessed by ALS functional rating scale (ALS-FSRS) and muscle strength by Manual Muscle Testing (MMT). Nine healthy subjects matched for age and educational level were assumed as controls. All subjects were tested by a neuropsychological battery including Mini Mental State Examination (MMSE), Prose Memory, Working Memory, Stroop Color Word Interference, Symbol Digit Modalities (SBM), Verbal Fluency and Beck depression inventory. MRI examination was performed by 1.5 Tesla GE. Coronal, axial T2 and 3D T1 weighted sequences were acquired. Total and regional brain atrophy was measured by VBM, a technique implemented in SPM99 consisting in a voxel-to-voxel parametric statistical test of 3D MR images after normalization, segmentation, smoothing and modulation.

Results: In ALS the score of ALS FSRS was 28.7 ± 7.2 and MMT 7.82 ± 1.02. MMSE and neuropsychological tests evaluating temporal lobe functions did not differ in ALS and controls. Tests evaluating frontal lobe functions showed significant low scores in SBM test (ALS 27.85 ± 14.22 vs. controls 38.57 ± 10.66, $p = 0.037$) and pathological values were found in 10/14 ALS in comparison to 1/9 controls. T2-hyperintensity at subcortical white matter was found in 12/14 ALS and only in one control. T2 low signal intensity at the precentral gyrus was identified in 3 ALS patients. Total gray and white matter volumes did not differ in two groups. Compared to controls, ALS showed a decrease of gray matter volume in temporal-hippocampal gyrus bilaterally ($p = 0.001$), in right superior ($p = 0.004$) and medium ($p = 0.008$) frontal gyrus and in left inferior frontal gyrus ($p = 0.009$). A significant correlation was found between frontal-orbital volume and SBM test ($p = 0.0001$).

Conclusions: This study shows more sensitivity of VBM analysis to detect a frontal and temporal dysfunction beyond the motor system.

O55

Heterozygous R1101K mutation of the DCTN1 gene in a family with amyotrophic lateral sclerosis and frontotemporal dementia

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The molecular mechanisms underlying the co-occurrence of ALS and FTD remain incompletely understood. We report a heterozygous R1101K mutation of the p150 subunit of the molecular motor dynactin (DCTN1) in a family with amyotrophic lateral sclerosis (ALS) and co-occurrence of frontotemporal dementia (FTD). Three members of our kindred were affected with motor neuron disease and two with dementia in an autosomal dominant pattern of inheritance. We excluded the involvement of the ALS and FTD-linked genes for copper/zinc superoxide dismutase (SOD1) and tau. The present study shows that mutations in DCTN1 may result in a wider range of phenotypes than has been previously appreciated. In this case, mutations in DCTN1 may predispose different neuron types to degeneration and then other genetic or external factors are required to produce the variable clinical phenotype. Taken together, our findings contribute to the current concept that familial FTD and ALS represent complex diseases, in which modifying genes and environmental agents can contribute to the risk of disease.

Session 8

Motor neuron disease 2

O56

Juvenile ALS with long-term survival associated with spastin gene mutation

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Background: Juvenile ALS (JALS) is a form of chronic motor neuron disease presenting with upper and lower motor neuron symptoms prior to the age of 25 years. Rare cases of JALS with a survival of more than three decades have been described. Genetic risk factors of sporadic JALS are largely unknown.

Objective: to describe a male patient with apparently sporadic JALS at the age of 72 years with a natural history of ALS for 48 years.

Design: a case report, magnetic resonance imaging of the brain and mutation analysis of the spastin gene (SPG4).

Result: at the age of 24 years the patient developed a progressive lower motor neuron syndrome of the left hand followed by paresis and atrophy of the distal left lower limb. In the course of 2 years he showed a pyramidal syndrome of all extremities and a progressive bulbar and pseudobulbar syndrome. Since then he has fulfilled the diagnostic criteria for definite ALS. Recent magnetic resonance imaging of the brain has demonstrated severe occipital, parietal and insular atrophy in decreasing order. Mutation analysis of the locus SPG4 for hereditary spastic paraplegia (HSP) identified a heterozygous protein-changing mutation (g 0.525_530dupGCCTCG) within exon 1 of the spastin gene.

Conclusion: We report the first case of ALS demonstrating a mutation in the spastin gene. We propose that sequence variants of spastin might serve as a previously unknown genetic risk factor for JALS. The finding implicates the potential involvement of the spastin gene in a greater spectrum of motor neuron disorders including clinical variants of ALS.

O57

Perturbation of mitochondrial anti-oxidant defence in a cell-culture model of SOD1-related familial amyotrophic lateral sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder. Whilst the majority of ALS occurs sporadically, ~10 % is familial. Of this inherited group, 20 % of cases are due to muta-

tions to Cu Zn superoxide dismutase (SOD1). There is increasing evidence that mitochondrial dysfunction may be important in the pathogenesis of both SOD1 familial ALS and the sporadic form of the disease.

Objectives: 1) To identify changes in mitochondrial protein expression attributable to the presence of mutant SOD1 in our cell-culture model of SOD1 familial ALS. 2) To establish the functional consequences of these changes and if possible demonstrate relevance to ALS in patients.

Methods: NSC34 cells are immortalized, motor neurone-like cells. Mitochondrially-enriched preparations of NSC34 cells stably transfected with empty transfection vector, normal human SOD1 or G93A mutant human SOD1 were made and 2-D SDS-PAGE performed. Silver-stained gels were analyzed using Phoretix software to identify differentially expressed protein spots. Changed spots were identified using MALDI-TOF mass spectroscopy and online database searching. Confirmatory Western blotting was performed in NSC34 cells and in G93A SOD1 transgenic mouse spinal cord.

Results: Of ~1200 protein spots appearing on each gel, the expression of 30 changed in a mutant SOD1 specific manner. These included anti-oxidant enzymes, apoptotic effectors, metabolic enzymes and components of the electron transport chain. Peroxiredoxin 3 (Prx 3), a thioredoxin-dependent hydroperoxidase, was shown to be down-regulated in mutant SOD1-expressing NSC34 cells and was also shown to be down-regulated in SOD1 transgenic mouse spinal cord. Data from the pharmacological manipulation of mitochondrial anti-oxidant defence will also be presented.

Conclusions: Mitochondria are particularly vulnerable to damage from reactive oxygen species generated by the electron transport chain. Despite this they lack catalase and express glutathione peroxidase at a level 30 fold below that of Prx 3. Given the evidence for oxidative stress in ALS, it is interesting that Prx 3, a mitochondrial matrix protein which might be expected to protect the mitochondrion from oxidative stress, is down-regulated. Changes in mitochondrial anti-oxidant defence may play a role in the death of motor neurones in SOD1-related fALS and the modulation of mitochondrial anti-oxidants may therefore offer therapeutic opportunities.

O58

A review of clinical trials in ALS: what is the role of clinical and neurophysiological measurements?

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Background: Since most of the Randomized Clinical Trials (RCTs) have been negative in Amyotrophic Lateral Sclerosis (ALS) and the mouse model has not given good insight about effective drugs, the study of a considerable number of compounds in small exploratory clinical trials could be an interesting alternative. For this last strategy, it is essential to choose very sensitive measurements which could give information about effectiveness in a small homogeneous group of patients.

Methods: To study the potentialities of different surrogate markers two authors independently reviewed all full published RCTs in ALS. We analysed the trial design, the number of patients enrolled, the primary and secondary end-points, the target change of the primary end-point and the statistical methodologies used. We selected for further analysis all placebo-controlled, parallel, double-blind RCTs, carried out over at least 6 months, with more than 29 patients per arm. Then we applied those measurements to calculate the sample size, based on their observed statistical features.

Results: We identified 56 RCTs in ALS. We excluded 33 which did not fulfil all of our inclusion criteria mainly because these were small studies carried out over a short period of time or used a cross-over or a non-double-blind design. From 24 RCTs chosen we extracted for the placebo arm the mean value and standard deviation at entry and the decline over 6 months of selected clinical measurements: Appel, Norris and ALS-FRS functional scales; manual muscle testing (MMT), maximal voluntary contraction and forced vital capacity. In addition, from these trials and personal data (group of 30 patients), we analysed the decline of M-wave amplitude, motor unit number estimation and neurophysiological index (NI). We calculated the number of patients per arm needed to observe a change of 50 % in the rate of progression (power = 80 %, alpha f n = 5 %) considering different measurements as primary end-points. In this model ALS-FRS (n = 67), MMT (n = 95) and NI (n = 122) seemed the most promising measurements.

Conclusions: In our model the most sensitive measure was ALS-FRS which is a simple rating scale with the advantage of the possibility of being employed by phone. Interesting neurophysiological measures were not as sensitive as we first thought because of patient inter-variability.

O59

Incidence and trends of amyotrophic lateral sclerosis in a population-based register

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Objective: To define the incidence and trends of amyotrophic lateral sclerosis (ALS) in Lombardy, Italy, during the period 1998–2002 through a population-based register (SLALOM).

Background: The incidence and trends of ALS have been mostly assessed in small population samples. Data are limited from well-defined large populations at risk.

Design/Methods: This study was performed in 8 provinces of Lombardy, total population 3,838,778. Enrolled were patients with newly diagnosed ALS during the years 1998–2002. The main source of case ascertainment was a prospective regional register of ALS, based on a network of neurologists from neurological, neurophysiological and rehabilitation units. Cases were also traced through the Hospital Discharge Diagnosis (HDD) code 335.2 (ALS) at the Central Regional Archive. For each eligible patient, the caring physician was required to collect the main demographic and clinical information in a semi-structured form. All forms were reviewed by a panel of experts and each eligible patient was classified according to a combination of the original and revised El Escorial diagnostic criteria. Overall crude, age and sex-adjusted mean annual incidence rates were calculated for the entire study population and for each year and province separately. Age specific incidence rates were also provided. Age and sex-adjustment was made with the direct method of standardization using the 2001 Italian population for reference.

Results: During the study period we collected a total of 392 patients (205 male, 187 female; M:F ratio 1.1) aged 22–92 yr at diagnosis (mean 63.4, SD 12.4). Onset of symptoms was bulbar in 30.8 % of cases and mixed (bulbar and spinal) in 6 %. ALS was definite in 50.9 %, probable in 26.4 %, probable laboratory supported in 2.7 %, possible in 18.6 %, and suspected in 1.4 %. Mean disease duration at diagnosis was 9.6 months. The crude incidence ratio was 1.98 per 100,000 in the year 1998, 2.08 in 1999, 2.01 in 2000, 2.42 in 2001, and 1.72 in 2002. There was a moderate variation of the incidence of the disease across provinces, which was mostly explained by the small numbers in some geographic areas. The standardized incidence ratio was 1.9 per 100,000/year (95 % CI 1.46–2.37).

Conclusions: This study confirms a steady incidence ratio of ALS over a 5-year period, without evidence of temporal and geographic clusters.

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O60

A phenotype-genotype study in late onset Spinal muscular atrophy types III and IV

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Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterised by progressive weakness in a limb girdle pattern. SMA is caused by a homozygous deletion of the survival motor neuron (SMN1) gene. Clinical phenotypes show variation with respect to age of onset, leading to distinction of 4 SMA types. SMN2, the centromeric copy of SMN1, is functionally less effective and can not compensate SMN protein deficiency. However, the SMN2 gene copy number may determine the clinical phenotype since high SMN2 copy numbers have been shown to correlate with a less severe phenotype and delayed onset. We did a prospective study of the disease course and genotype-phenotype correlation of late onset SMA types III and IV.

Methods: Twelve patients fulfilled the diagnostic criteria for SMA. SMN2 copy number was determined using a quantitative competitive PCR method. Patients were screened at inclusion and after a mean of 30 months (range 19–36). We did manual muscle testing (MMT) using the medical research council (MRC) of 46 muscles, functional status (Amyotrophic Functional Rating Scale (ALSFRS), and quality of life (Short Form Health Survey (SF-36).

Results: Genotype All patients had a homozygous deletion of exons 7 and 8 of the SMN1 gene. Eleven patients had 5, and 1 patient had 6 SMN2 copies. Seven patients were male, 5 female. Six patients were siblings from family A. Two patients were brothers (family B), 4 patients were sporadic.

Phenotype Median disease onset was 28 years for all patients (range 13–37). Two brothers (age 12 and 16) from family A were asymptomatic. Disease onset in the affected sibs (family A) was earlier compared with other patients. At follow up MMT score decreased 12 to 17 points in 3 pa-

tients but remained stable in the others. ALSFRS and quality of life did not change.

Discussion: Muscle strength deteriorated in 3 patients, but without effect on functional status or quality of life in 12 patients with late onset SMA during a mean follow-up of 2.5 years. All patients carried 5 or 6 SMN2 copies. Interestingly, age of onset in the affected sibs was lower as compared with the other 6 patients. This observation may indicate that genetically undetermined factors, possibly outside the SMN locus, determine SMA phenotype and disease course. Using the described outcome measures, we could not demonstrate progression of disease at 2.5 years follow-up. This notion may be important for the design of future trials.

O61

Biological significance of the p38 mitogen activated protein kinase in a mouse model for ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the selective loss of motor neurons. In 20 % of these familial cases, mutations in the superoxide dismutase 1 gene (SOD1) have been reported. To date, no effective treatment is available and the pathogenic mechanism remains enigmatic.

We have previously reported minocycline to significantly attenuate mutant SOD1-induced motor neuron degeneration and to expand the life span of mutant SOD1 overexpressing mice, a mouse model for human ALS. It is thought that the effect of minocycline is exerted through an inhibition of the activation of the p38 mitogen activated protein kinase (MAPK) pathway, which is known to occur in microglia and motor neurons of mutant SOD1 mice.

In the present study, we aimed to characterize the biological relevance of upregulation of p38 MAPK for motor neuron degeneration in the mutant SOD1 mouse.

We found the p38 MAPK to be abnormally activated in the ventral part of the spinal cord of the mutant (G93A) SOD1 mice, where significantly increased levels of phosphorylated p38 MAPK were found. The therapeutic effect of minocycline was accompanied by a significant attenuation of this activation. Activated phospho-p38 MAPK was exclusively present in microglial cells and motor neurons. In vitro studies showed minocycline to inhibit microglial activation by lipopolysaccharide. In addition, both minocycline and SB203580, a specific p38 MAPK inhibitor, inhibited mutant SOD1-induced apoptosis in cultured motor neurons. We therefore tested whether semapimod, a specific p38 MAPK inhibitor that readily crosses the blood brain barrier, affected mutant SOD1-induced motor neuron death. Mutant (G93A) SOD1 mice were treated with semapimod or placebo from 70 days of age on. Treatment with semapimod effectively inhibited p38 MAPK activity, as phosphorylation of tau at residue Thr231 was significantly reduced. It significantly enhanced survival of mutant (G93A) SOD1 mice and attenuated motor neuron death in these animals. However, its effect was clearly smaller than that of minocycline.

In conclusion, our data suggest the p38 MAPK pathway to play a significant albeit limited role in mutant SOD1 induced motor neuron degeneration. As the effect of treatment of mutant SOD1 mice with semapimod is smaller than that observed with minocycline, the effect of the latter drug is likely to be mediated by other effects than inhibition of the p38 MAPK pathway alone.

Session 9

Multiple sclerosis 1

O62

Expression of the Treg cell associated FOXP3 gene is decreased in MS patients and increases following immunisation with a T cell receptor peptide vaccine

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Background: Multiple sclerosis (MS) may result from the failure of tolerance mechanisms that prevent expansion of pathogenic T cells that react against myelin antigens. These tolerance mechanisms include CD4+CD25+ Treg cells, which express the FOXP3 gene. Previous studies attempting to demonstrate abnormalities of Treg cell activity in MS using functional assays have given conflicting results.

Objective: To determine whether expression of FOXP3, a gene associated with CD4+CD25+ Treg cells, is decreased in MS patients and whether administration of a T cell receptor (TCR) peptide vaccine can increase FOXP3 expression.

Methods: CD4+CD25+ cells were isolated from peripheral blood mononuclear cells from 19 MS subjects entering an open label trial of a trivalent TCR peptide vaccine (NeuroVax®) and from 19 healthy controls. RNA isolated from the CD4+CD25+ cells was subjected to real time polymerase chain reaction to quantitate FOXP3 mRNA expression. Western blot analysis was also performed on a subset of these subjects to detect FOXP3 protein expression. FOXP3 mRNA in CD4+CD25+ cells from MS subjects also was determined 12 weeks after initiating immunisation with the trivalent TCR peptide vaccine.

Results: FOXP3 mRNA in CD4+CD25+ cells was decreased among MS subjects (n = 19) compared with healthy controls (n = 19) ($p = 0.02$). FOXP3 protein in CD4+CD25+ cells was also significantly diminished in MS subjects compared with healthy controls. Following administration of the trivalent TCR peptide vaccine, FOXP3 mRNA in CD4+CD25+ cells increased to levels comparable to that of healthy controls.

Conclusion: This is the first demonstration that FOXP3 expression is decreased in MS patients compared with healthy controls, suggesting impaired immunoregulation by Treg cells. Importantly, a trivalent TCR peptide vaccine appears capable of boosting FOXP3 expression and this may represent a novel treatment that can enhance Treg activity in MS.

O63

Regulatory T cells are highly susceptible to CD95L-induced apoptosis and display a loss of suppressive function in multiple sclerosis

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Regulatory T-cells of CD4+CD25+ FoxP3+ phenotype (Treg) suppress T-cell function and protect rodents from organ-specific autoimmune disease. The role of regulatory T cells in human autoimmune disease and its modulation are of major interest. Enhanced survival and expansion of regulatory T cells would be beneficial in autoimmune diseases such as Multiple Sclerosis, whereas increased depletion by apoptosis would be advantageous in cancer. In addition to their described sensitivity to cytokine deprivation, we show that freshly isolated FACS-sorted human regulatory T cells are highly sensitive towards CD95-dependent apoptosis in contrast to their CD4+CD25– T cell counterparts. However, restimulation of expanded regulatory T cells revealed a reduced sensitivity towards activation induced cell death (AICD) in contrast to AICD-sensitive CD4+CD25– T cells. Simultaneously, expanded regulatory T cells remained highly sensitive towards CD95L-triggered apoptosis. Murine CD4+CD25+ regulatory T cells displayed a similar sensitivity. Our data suggest a model in which CD4+CD25– effector T cells could modulate the number of regulatory T cells via the CD95L/CD95 system in the contraction phase of an immune response. Furthermore, we found a defective suppressive function of regulatory T cells in Multiple Sclerosis patients. Given known alterations of the CD95 system in MS we are investigating whether an altered sensitivity of regulatory T cells towards CD95-dependent apoptosis could be critical for the modulation of defective regulatory response in Multiple Sclerosis.

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O64

Mitochondria and axonal loss in multiple sclerosis: the shiverer mouse as a model of secondary progressive MS

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The aim of this study was to investigate the hypothesis that the upregulation of mitochondrial function plays an essential role in the maintenance of conduction in axons lacking myelin.

Axonal pathology in multiple sclerosis (MS) has been described for over a century but new insights into the relationship between axonal loss and the accumulation of neurological disability has focused renewed interest in this area of study. Several lines of evidence support the theory that

mitochondria play a role in MS pathology. An intriguing relationship appears to exist between the mitochondrial gene defects causing Leber's Hereditary Optic Neuropathy and an MS-like condition suffered by some of these patients. Impaired axonal mitochondrial function has been demonstrated in MR spectroscopy studies in vivo and evidence of oxidative damage to mitochondrial DNA has been demonstrated in chronic MS plaques.

The shiverer mouse model is a myelin basic protein mutant with dysmyelinated axons from birth. Brain and spinal cord histology was compared between shiverer and wild type (WT). Luxol fast blue and cytochrome c oxidase (COX) histochemistry was performed on serial sections. Time lapse experiments were performed to calculate the relative rate of COX activity between the shiverer and WT. Perfusion fixed tissue was processed for EM for the estimation of mitochondrial density.

COX activity was increased in the dysmyelinated axons in the spinal cord and corpus callosum of shiverer compared to WT. Time lapse experiments confirmed the rate of COX activity was 2 fold higher in shiverer spinal cord and 6 fold higher in corpus callosum compared to WT. EM studies demonstrated a 50 % increase in density of mitochondria within the hypomyelinated shiverer axons, confirming that the increased mitochondrial activity lay within the axons. Comparable results have been obtained in initial studies in human MS brain tissue with increased COX activity present in chronic demyelinated MS plaques compared with myelinated control tissue.

Our studies in the shiverer mouse show an increase in mitochondrial density and activity in axons lacking myelin and imply that there is an increased energy requirement in these axons. We suggest that this energy is required to maintain conduction and viability. Therefore, we hypothesise that axonal loss in MS may occur if mitochondrial function is compromised in vulnerable demyelinated axons.

O65

Quantitative real-time polymerase chain reaction and indirect immunofluoresence to validate the effects of mixtures of cytokines on gene and protein expression in mixed central nervous system glial cell cultures

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Cytokines secreted within the central nervous system (CNS) by infiltrating and endogenous cells are important in the pathogenesis of the multiple sclerosis (MS) lesion and also likely to influence inhibition of the lesions as well as reparative mechanisms. To examine in vitro some of the molecular and cellular processes involved we examined early gene expression in mixed CNS glial cultures in response to mixtures of Th1, Th2 and monocyte/macrophage (M/M) cytokines employing microarray technology. Using replicate cultures we observed differential regulation of expression of 841 genes by one or more of the cytokine mixtures when compared to control cultures using criteria of changes of 2 fold or greater ($p < 0.2$). To begin to examine genes and proteins of interest and to validate using mixtures of cytokines and mixtures of glial cells we performed quantitative real time polymerase chain reaction (QRT-PCR) on RNA extracted from cultures stimulated with the different cytokine mixtures compared to controls. We also studied the effect on 2 proteins after 3 days of stimulation employing indirect immunofluoresence (IF) using specific antibodies to these proteins, identifying the different cell types using antibodies to phenotypic markers. At 6 hours Th1 cytokines induced a 50 fold upregulation in the gene for CD74, the invariant polypeptide of major histocompatibility complex (MHC) class II, when compared to a housekeeping gene, Th2 and M/M had no effect. Th1 and M/M upregulated the gene for intercellular adhesion molecule-1 (ICAM-1) 13 and 6 fold respectively. Th2 cytokines had no effect. Th2 and M/M cytokines upregulated the gene for osteoprotegerin (OPG), a member of the tumor necrosis factor receptor superfamily, 13 and 3 fold respectively. Th1 had no effect. These results confirm our observations with these genes using microarrays. We detected upregulation of MHC class II by IF predominately on microglia and to a lesser extent on astrocytes in response only to Th1 cytokines but no effect on oligodendrocytes (OLG) or OLG precursors (OPC). ICAM-1, which was constitutively expressed by astrocytes and microglia, was further upregulated on astrocytes and probably by microglia by all 3 cytokine mixtures with no effect on expression in OLG or OPC. Our findings with QRT-PCR and IF support the use of microarrays to screen effects of cytokine mixtures on gene expression as well as subsequent effects on proteins employing mixed CNS glial cell cultures.

O66

Molecular analysis of normal-appearing white matter in multiple sclerosis

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MS is a chronic inflammatory disease of the CNS leading to focal destruction of myelin. However, the earliest changes that lead to lesion formation are not known. Our recent microarray analysis of normal-appearing white matter (NAWM) revealed upregulation of a number of functionally related genes known to be involved in endogenous neuroprotection as well as in maintenance of cellular homeostasis (Graumann et al., 2003). Our results introduced a novel concept for the molecular pathogenesis of MS with ischemic preconditioning as an important mechanism for neuroprotection. To identify the cellular expression pattern of some representative genes we performed in situ hybridization and quantitative real-time PCR revealing that HIF1alpha as the key transcription factor is upregulated in NAWM in the majority of MS cases. The question arises, which of these alterations occur in the early stage of MS, and which are the consequences after long disease progression. Biopsy tissues are routinely embedded in paraffin, and therefore, we have established a protocol for successful isolation of total RNA allowing qRT-PCR and detection of low abundant genes such as HIF1alpha. In an ongoing study, we have characterized NAWM from a needle biopsy of a 18-year old female patient. Immunopathological examination revealed NAWM with relatively sharp bordered lesion with dense infiltrate of foamy transformed macrophages as well as perivascular accentuated lymphocytes. Within the lesion there were beside oligodendrocytes with normal appearance also many oligodendrocytes with dark and fragmentized nuclei suggestive of apoptosis. Further, the specific absence of MAG in contrast to PLP, CNP and MOG in myelin debris within foamy macrophages was evident. Altogether, this pathology is in agreement with Pattern III lesions after the classification by Lucchinetti and Brück (2002) suggesting that these lesions develop under conditions of a hypoxia-like type of tissue damage. We isolated total RNA from NAWM tissue samples of this patient and performed qRT-PCR as well as microarray analysis. First results of this study exhibit strong expression of myelin genes such as MBP, MOG and PLP reflecting the ongoing myelination process at that young age. We detected upregulation of HIF1alpha in NAWM, which is in keeping with the fact that this expression factor may be an important protective factor in preventing lesion formation. Further studies are currently performed to confirm these findings.

O67

P-selectin (SELP) and P-selectin glycoprotein ligand-1 (SELPLG) polymorphisms in multiple sclerosis patients

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P-selectin (SELP) and P-selectin glycoprotein ligand-1 (SELPLG) constitute a receptor/ligand complex involved in the recruitment of activated lymphocytes, a critical event in the pathogenesis of multiple sclerosis (MS). This process is dependent upon adhesion molecules, in particular SELP on cerebral endothelium and its ligand SELPLG on lymphocytes. A novel Single nucleotide Polymorphism (SNP), involving a G to A substitution, resulting in a non-synonymous amino acid change from Methionine (Met) to Isoleucine (Ile) at position 62 of the coding region has been described as well as an A to C variation in the SELP gene resulting in a change from a polar amino acid for a non-polar one at the exon 13 (Thr715 Pro).

In order to determine whether genetic variation in these pivotal genes influences susceptibility to MS two caucasian populations were typed.

DNA was isolated from peripheral blood leukocytes by standard procedures. The Met62Ile determination was first performed on an Italian population of 200 patients with MS as well as on 200 controls with a new PCR-RFLP method validated by sequencing. Subsequently 938 UK trio families have been typed for the Met62Ile variant in SELPLG and the Thr715Pro variant in SELP using the Taqman 5'-nuclease assays. All patients met Mc Donald's criteria for the diagnosis of MS. A significantly decreased allelic frequency of the Met62Ile variant in MS patients compared with controls was observed (8.0 vs. 13.9 %, $p = 0.03$) in the Italian population. In order to confirm this preliminary result, a bigger population constisting of 938 trio families (an affected index patient and both parents) was typed for the same variant and for the Thr715Pro SNP. Neither of these SNPs showed evidence for association with susceptibility to MS.

This study represents the first attempt to screen a population for the genetic variants Met62Ile and Thr715Pro in the SELPLG and SELP genes re-

spectively. Furthermore different technical approaches were used to this purpose, and a novel PCR-RFLP method to detect the Met62Ile variant was developed.

The decreased frequency of the Met62Ile observed in the Italian MS population was not confirmed in the wide UK population screen thus suggesting that none of the SNPs analysed is associated with susceptibility to MS. However, other polymorphisms in the same genes need to be explored to conclusively exclude SELP and SELPLG as risk factors in genetic predisposition to MS.

Session 10

Multiple sclerosis 2

O68

Brain magnetisation transfer MRI reflects clinical changes over 18 months in patients with relapsing-remitting multiple sclerosis

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Background: Magnetization transfer (MT) MRI is a non-conventional MRI technique, which can provide quantitative estimates of microscopic tissue damage in normal-appearing white (NAWM) and gray (NAGM) matter from patients with MS.

Objective: To assess whether MT MRI at a given time point is associated to short-term disease evolution.

Methods: Twenty-six patients with clinically definite MS and a RRMS course were studied over an 18 months follow-up period. None of the patients had had immunosuppressive or immunomodulating treatments before or during the observation period. Clinical follow-up comprised neurological visits with expanded disability status scale (EDSS) rating every three months. Brain MR measurements were performed on a 1.5 T scanner at baseline. The following images were acquired: 1) dual-echo turbo spin echo (SE); 2) 2D gradient echo with and without a saturation MT pulse; and 3) T1-weighted SE 5 minutes after the injection of 0.1 mmol/kg of gadolinium-DTPA. MTR histograms from the whole brain tissue, the NAWM and the NAGM were produced.

Results: During the follow up, 16 patients (61 %) experienced 36 relapses. The median EDSS scores were 1.5 (range: 0.0–4.0) at study entry and 2.0 (range: 0.0–4.0) at study exit. At final visit, seven patients (27 %) were considered clinically worsened based on EDSS score deterioration. Average whole brain ($r = -0.438$; $p = 0.03$) and NAGM ($r = -0.36$; $p = 0.03$) MTR values at baseline were correlated with EDSS changes over 18 months. The latter changes were, on turn, significantly correlated with the number of relapses during the follow-up period (r value: 0.482; p: 0.02)

Conclusions: This study shows that a 'snapshot' MT MRI assessment detects subtle brain tissue changes which are associated with short-term disability accumulation. This sounds promising for the use of MT MRI metrics as paraclinical markers of MS evolution. (Supported by a grant of ENS)

O69

Longitudinal changes in T2 lesion volume in multiple sclerosis: possible restricted utility as a clinical trial outcome

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The large multinational database of the Sylvia Lawry Centre for Multiple Sclerosis Research (SLCMSR) currently consists of 85 % of all available multiple sclerosis placebo patients observed in clinical trials of the past 15 years. One use of this database is to contribute to the discussion on the usefulness of various magnetic resonance imaging (MRI) measures as possible surrogate markers for future trials. The aim of this study was to determine factors that influence the longitudinal relationship between MRI changes of T2 lesion volume over time and a set of clinical and demographic variables.

As MRI activity over time varies more between patients than within patients, we used a statistical model that individually adjusts for this inter-patient variation through random effects. Additionally, we adjusted for heterogeneity between centralized image analysis centres.

Potential predictor variables measured at entry into the clinical trials included gender, the number of relapses in the two years before trial entry, age at disease onset, and disease course. Others, like the EDSS scale, disease duration, and gadolinium enhancement status vary over time on study. We extracted from the open portion of the SLCMSR database complete data on 816 patients with 3783 longitudinal observations over a median observation time of two years for this analysis.

Variable selection was necessary due to inter-correlation between the predictors. After adjustment for the effect of the individual image analysis centre, EDSS and disease duration emerged as the variables that remained in the model to explain T2 lesion volume. We found the relationship to be non-linear, with positive but weak correlation in the early years after MS onset, and a plateauing relationship of T2 burden of disease for patients having MS for more than 10 years. This association confirms earlier cross-sectional analyses from the Sylvia Lawry database. The positive association between EDSS, disease duration, and T2 lesion volume for MS patients with short to medium disease durations could have important implications for the design of future clinical trials that consider the change in T2 lesion volume as an outcome. The confirmation of the plateauing effect in this longitudinal analysis suggests that the utility of T2 lesion volume as a potential surrogate marker for disability may be limited to selected cohorts of patients only within well defined phases of the disease.

O70

A longitudinal, frequent MRI study of grey matter volume changes in active relapsing-remitting multiple sclerosis

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Objective: To investigate the patterns of short-term evolution of grey matter (GM) volume in a large sample of patients with relapsing-remitting (RR) multiple sclerosis (MS) by assessing the magnetic resonance imaging (MRI) dataset from the placebo arm of the European/Canadian glatiramer acetate (GA) trial.

Background: Several pieces of evidence suggest that conventional MRI-undetectable GM damage occurs soon after the clinical onset of MS. Little is known, however, about the time course of this damage, as well as about its relationship with other MRI-derived markers of MS evolution.

Design/Methods: The European/Canadian GA trial was a nine-month, double-blind, placebo-controlled study, where 239 RRMS patients were randomized to receive either 20 mg GA ($n = 119$) or placebo ($n = 120$) by daily subcutaneous injections. Dual echo, pre- and post-gadolinium (Gd) T1-weighted MRI scans of the brain were obtained at screening (to be included, patients had to have one or more enhancing lesions), baseline and every month during the follow-up period. Active lesions were counted and total T2-hyperintense and T1-hypointense lesion volumes (LV) measured. Using the Structural Imaging Evaluation of Normalized Atrophy (SIENA) software and its cross-sectional version (SIENAX), normalized whole brain tissue (WBT), GM and white matter (WM) volumes were computed from all monthly scans and two-timepoint percentage brain volume change (PBVC) was measured between baseline and month 9 scans.

Results: Data from 113/120 placebo patients were available for the present analysis. Over the nine months of the study, the mean PBVC for WBT at month 9 vs. baseline was –0.93 % (standard deviation (SD): 1.16) and the mean percentage changes of GM and WM volumes were –2.30 % (SD: 4.78 %) and 0.57 % (SD: 4.86 %), respectively. Time-trend analysis revealed that GM volume decrease was statistically significant ($p < 0.001$). A random effect model correlation analysis showed that there was a significant relationship of GM decrease with both T2 LV and T1 LV increases over the same period of time (p values were 0.038 and 0.026, respectively).

Conclusions: This study confirms that significant GM volume decrease occurs over short periods of time in patients with RRMS selected for MRI evidence of ongoing inflammatory activity. The progression of GM atrophy seems to depend, at least partially, upon the concomitant accumulation of MRI-visible white matter damage.

O71

Magnetisation transfer histogram abnormalities in clinically isolated syndromes suggestive of multiple sclerosis

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In established multiple sclerosis, magnetisation transfer ratio (MTR) histograms reveal abnormalities of normal appearing white matter (NAWM) and grey matter (NAGM). The aim of this study was to investigate the stage at which these changes first occur by looking at patients presenting with clinically isolated syndromes (CIS) suggestive of multiple sclerosis.

Methods: MT imaging was performed on 100 CIS patients (67 women, 33 men, median age 32, median EDSS 1; 89 optic neuritis, 6 brainstem and 5 spinal cord syndromes) with a mean delay of 19 weeks after symptom onset and in 50 healthy controls (34 women, 16 men, median age 32.5). A 2D spin echo sequence was used on a 1.5 Tesla scanner using a repetition time of 1730ms and echo times of 30/80ms. SPM99 software was used to generate segmented NAWM and NAGM MTR maps. Partial volume effects were minimized using a 10pu (percent units) lower threshold and voxel erosions. MTR histograms, normalised for brain volume, were generated with a bin width of 0.1pu. In-house software was used to calculate the volume of T2 lesions, WM and grey matter. Multiple regression was used to look for differences between patients and controls with age, gender and volume measures as covariates to control for potential confounding effects.

Results: The MTR histograms for both CIS NAWM and NAGM showed a reduction in mean MTR (NAWM, 38.33 vs. 38.14, $p = 0.001$; NAGM 32.50 vs. 32.29, $p = 0.009$; units in pu) and histogram peak location (NAWM, 38.52 vs. 38.37, $p = 0.011$; NAGM 33.35 vs. 33.18, $p = 0.015$), with a left shift in the histogram as indicated by a reduction in MTR at the 25th, 50th and 75th percentiles. The peak height of the histogram was not significantly reduced.

Significant MTR abnormalities were seen in the subgroup of 77 CIS patients with abnormal T2-weighted brain MRI and in the 25 who satisfied the McDonald criteria for MS. CIS patients with normal T2-weighted brain MRI did not show significant abnormalities. NAWM MTR correlated with white matter and grey matter fractional volume and NAGM MTR correlated with the volume of T2 lesions, white matter fractional volume and brain parenchymal fractional volume.

Conclusions: This study provides evidence for subtle MTR abnormalities occurring in the NAWM and NAGM at the earliest clinical stages of MS. Follow up studies are underway to determine whether these abnormalities in the normal appearing brain tissue are related to the subsequent clinical evolution of the disease.

O72

Effects of immunomodulatory drugs on regulatory T cells in multiple sclerosis patients and healthy individuals

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Background and Aims: Regulatory T cells (Treg) appear to play a crucial role in the pathogenesis of autoimmune diseases such as multiple sclerosis (MS), although their mechanism of action is not yet understood. Recent studies have shown diverse effects of drugs on Treg, either influencing their content of FoxP3 mRNA, surface-marker pattern or their longevity. However, there are few data concerning the influence of drugs on their inhibitory capacity. Data elucidating the changes that Treg might undergo in peripheral blood of patients receiving long term treatment with immunomodulatory drugs are equally scarce, as these drugs act upon both diverse lymphocyte subsets and antigen presenting cells.

To address this issue the effects of immunomodulatory drugs upon different T cell subsets were examined both in vitro and in vivo.

Subjects: Patients had definite relapsing remitting MS or secondary progressive MS. All patients were treatment naïve. Serial testing was performed in patients commencing long term treatment with immunomodulatory drugs. Treg from healthy controls were examined for drug effects in vitro.

Methods: Treg and Teff were separated from peripheral blood samples by immunomagnetic isolation. Purity and phenotype of cell fractions were assessed by flow cytometry. Suppressive function of Treg was tested by primary proliferation assays. For analysis of in vitro drug effects Treg or Teff were pre-incubated separately with interferon beta, glatiramer acetate, 17-beta estradiol, corticosteroids or transforming growth factor (TGF)-beta.

Results and Conclusions: In vitro TGF-beta significantly improved the inhibitory capacity of Treg, the effects of 17-beta estradiol were, although positive, less marked. The effect was lower when incubating Teff with these drugs. Interferon-beta and glatiramer acetate influenced the regulative capacity as well as the responsiveness of Teff. Treg obtained from MS patients before and following treatment with immunomodulatory drugs exhibited enhanced suppressive effects in the majority of cases as compared to inhibition before therapy.

Thus, the targeted pharmacologic modulation of Treg activity may be of significant clinical importance in the treatment of MS and other autoimmune diseases.

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O73

Interferon-beta leads to stabilisation of barrier characteristics in brain capillary endothelial cells from four different species in vitro

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Objective: To test whether the influence of interferon-beta (IFN-beta) on the barrier function in brain capillary endothelial cells (BCEC) in vitro is consistent in different species.

Background: Blood-brain barrier (BBB) breakdown is an early event in the pathogenesis of multiple sclerosis (MS) or its animal model experimental autoimmune encephalomyelitis. Besides clinical benefits, serial MRI scans from IFN-beta treated MS patients show a reduction of Gadolinium-enhancing lesions. In a previous study we found a direct stabilization of barrier characteristics after treatment of bovine BCEC with IFN-beta in an in vitro BBB model.

Methods: We applied different in vitro models consisting of BCEC from four different species. We used primary bovine and porcine BCEC, whereas human and murine BCEC were from immortalized endothelioma cell lines. We investigated the influence of human recombinant IFN-beta on the paracellular permeability for 3H-inulin and 14C sucrose across monolayers of human and murine BCEC as well as of bovine BCEC which had been grown in co-culture with astrocytes. In addition, the transendothelial electrical resistance (TEER) was determined in in vitro systems applying porcine and murine BCEC.

Results: In all applied in vitro models we found a stabilization of barrier characteristics after pretreatment with IFN-beta: Addition of IFN-beta resulted in a significant decrease of the paracellular permeabilities across monolayers of human, bovine and murine BCEC. Furthermore, the TEER was significantly increased after pretreatment of porcine and murine BCEC with IFN-beta.

Conclusion: We found that IFN-beta derived stabilization of BBB characteristics was consistent applying two different methods as well as primary and immortalized BCEC from four different species. This species-independent stabilizing effect of IFN-beta on BCEC in vitro may contribute significantly to the beneficial effects of IFN-beta treatment in MS in vivo.

Session 11

Muscle and neuromuscular junction disorders

O74

HLA typing in myasthenia gravis patients in Saudi Arabia

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Objective: To study the association of MG with HLA antigen in Saudis. Background: MG is an autoimmune disorder involving NMJ receptors. The diagnosis usually made by the characteristic symptoms and signs, edrophonium (Tensilon) test, electrophysiology studies and acetylcholine receptor antibody test. There is a moderate association of MG with the human leukocyte antigen (HLA) system that are coded for by MHC genes. MG associated with HLA system varies in different ethnic groups. In Caucasians, MG associated with the HLA antigens B8, DR3 and DQw2, particularly in young females with thymic hyperplasia. However, HLA association is different in Japanese and French patients. Pilot study in Saudi MG patients suggested association with HLA DQ5 and B18.

Design/Methods: Forty two patients from different families with confirmed diagnosis of MG and 345 normal controls were involved in this study. Genomic DNA was extracted using Puregene kit (Gentra Systems) and HLA typing was performed using PCR SSP kit (Dynal). Statistical testing for association was preformed using Epi Info software system. Odds Ratio (OR) was calculated with 95% confidence interval. P value < 0.05 is considered significant.

Results: There is significant association noted in Saudi MG patients on the following HLA type: HLA DQ 5 (P < 0.0001, OR 4.09, CI 1.9–8.81), HLA B8 (P < 0.003, OR 3.77, CI 1.38–5.49) and HLA B18 (p < 0.006, OR 93.77, CI 1.37–10.3)

Conclusions: HLA DQ5, HLA B8 and B18 are associated with MG in Saudi patients. AS noted in other ethnic groups HLA B8 is associated with MG in young females.

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O75

Prognosis after thymectomy in autoimmune myasthenia gravis

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Thymectomy in autoimmune myasthenia gravis (MG), is recommended to increase probability of improvements and remission. However, there is debate regarding evidence of benefit especially in elderly and in non thymomatous patients.We reviewed our experience to identify predictors of outcome in patients thymectomized for thymoma, for non neoplastic thymus and in those not treated surgically. A long term observational study of 72 months was conducted on 93 MG patients (mean age 65.6 years; range 14–82): 40 were males (43.0%; mean age 67.0), 53 females (57.0%; mean age 64.5). Mean duration of illness was 93 months, range1–537 months. Disease severity was defined according to Osserman's and Myasthenia Gravis Foundation of America clinical classifications. Onset of symptoms and signs was defined "early" for patients aged less than 60, "late" when age was equal or more than 60 years. 36.5% of patients (N = 34; mean age 45.2; range 15–77) were thymectomized for thymomas (N = 21), for non neoplastic thymus (N = 13). 63.4% of patients were not surgically treated. At diagnosis, 39 patients (42.0%) were classified in grade I, 31 (33.3%) in IIa, 23 (24.7%) in IIB; no patients were asymptomatic, neither in stage III nor IV. According to "actual"severity, 7 patients (7.5%) were asymptomatic, 40 (43.0%) were in stage I, 34 (36.5%) in stage IIa, 12 (12.9%) in IIb. Among thymectomized, 26 (76.5%) patients had onset below age of 60 years, 8 (23.5%) were aged equally or more than 60. Clinical outcome was evaluated in patients who underwent surgery for thymoma (22.6%), for non neoplastic thymus (14.0%) and in those not treated surgically (63.4%). Statistical analysis of our cohort of MG patients showed as follows:

1. at diagnosis, degree of impairment significantly differed in the three observational groups. Thymectomized patients were more severely affected (52% of patients graded as IIb had thymomas, 26% non neoplastic thymus; 92% among those not treated surgically were graded as I (chi square 32.4378; df 4; p < 0.0000016).
2. Thymectomy raised chance of improvement. Percentages of subjects that improved were 14% among non thymectomized, 32% among thymectomized patients (chi square 4.6865; df 1; p < 0.0304).
3. Patients who benefited from thymectomy were more often those wth non neoplastic (62%) than with neoplastic (14%) thymus (chi square 11.0285; df 1; p < 0.0009).
4. Thymectomy did not influence significantly outcome in early as compared to late onset MG (chi square 0.8850; df 1; p ns).

O76

CD4+CD25+ regulatory T cells are functionally deficient in patients with myasthenia gravis

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Thymus-derived CD4+CD25hi T regulatory cells play a decisive role in the generation and maintenance of peripheral tolerance. These cells are naturally hyporesponsive in vitro and can inhibit the proliferation of co-cultured CD4+CD25– conventional T cells in a contact-dependent manner. Although regulatory T cells have been largely involved in the prevention of autoimmune diseases in animals, only limited information is available on their role in the pathogenesis of human autoimmune diseases. We first analyzed the frequency of CD4+CD25hi in peripheral blood and in the thymus/thymomas of patients with myasthenia gravis (MG), a prototype autoimmune disease mediated by autoantibodies against the acetylcholine receptor at the neuromuscular junction. The frequency of regulatory cells was not altered in the peripheral compartment, but we found a marked decrease in the number of CD4+CD25+ thymocytes in MG-associated thymoma, suggesting that the thymic development of regulatory T cells is impaired in these patients. By contrast, patients with myasthenia-associated thymic hyperplasia showed nearly normal levels of CD4+CD25+ cells. We hypothesized that altered functions of peripheral CD4+CD25 hi regulatory T cells in the periphery play a role in the breakdown of immunologic self-tolerance in patients with MG. Suppression assays were thus conducted to analyze the functionality of these regulatory cells in vitro. We report a highly significant decrease in the suppressive function of the CD4+CD25hi cells from peripheral blood of patients with myasthenia gravis in comparison to healthy donors. The maximum defect in the suppressive function was observed in untreated patients, while patients that were under cytostatic or immunosuppressive drugs showed only a mild decrease in function. One important question that arises now is why the regulatory T cells in patients with autoimmune disease are not functional. Our current investigations aim to establish the role of steroid hormones in the control of the regulatory function by these cells.

O77

Specific cognitive changes in myotonic dystrophy type 1 and proximal myotonic myopathy compared with healthy controls

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Backround: Myotonic dystrophy types 1 (DM1; Curschmann-Steinert) and 2 (DM2; proximal myotonic myopathy; PROMM) are both inherited multisystem disease. Mean clinical features are neuromuscular symptoms (weakness, myotonia) and a range of potential systemic manifestations (ear, eye, heart, endocrine organs), including a variety of cerebral abnormalities (atrophy, white matter lesions). DM1 is caused by an expansion of a CTG trinucleotide repeat tract of the dystrophia myotonica-protein kinase gene and DM2 is caused by an expansion of a CCTG repeat tract in the zinc finger protein 9 gene. Significant brain involvement in both diseases in prefrontal cortex has been shown by MRI, FDG-PET, SPECT and neuropsychological studies. But there is a lack of studies with clearly matched patients comparing DM1, DM2 with healthy controls.

Methods: We included 21 DM1, 21 DM2 of normal intelligence with moderate symptoms and early adult and adult onset and 21 matched controls. Diagnosis was performed clinically and by genetic diagnostic in all patients. We investigated the neuropsychological performance using tests of frontal lobe function: the Iowa Gambling Task (sensitive to decision making impairment following orbito-frontal lobe damage) and the conditional associative learning (CAL) (sensitive to dorso-lateral prefrontal lobe damage). Additionally we tested premorbid intelligence, visual construction, visual memory, attention, verbal fluency, and mood status.

Results: Both groups (DM1 and DM2) had very similar scores on specific testing but differ significantly from controls. Half of the patients showed deficits in Iowa Gambling Task. Conditional associated learning was impaired in both groups, more distinctive in DM1.

Discussion: The study confirmed previous published results of a specific cognitive profile in DM1/DM2. Our data suggest that DM1/DM2 are linked to impairment of frontal lobe function. When the groups are matched for education and intelligence DM1 and DM2 patients do not differ in cognitive impairment, as previously thought.

O78

Distribution of glucocorticoid receptor subtypes in inflammatory myopathy

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Background: Glucocorticoids (GC) are standard treatment for inflammatory diseases. Though most dermatomyositis (DM) and polymyositis (PM) patients are treated successfully, sporadic inclusion body myositis (sIBM) patients are generally non-responsive. Imbalance between the active alpha and the inactive beta isoform of the GC receptor (GCR) has been put forward as a possible cause of GC resistance in human inflammatory disease.

Methods: We investigated GCR distribution using polyclonal antibodies specific for GCR, GCRalpha and GCRbeta. Frozen sections from normal controls and IIM patients were assayed through immunohistochemical and immunofluorescent double stainings. Patients were either treatment-naive or treated with GC in conventional doses before the biopsies were taken.

Results: In all diagnostic groups GCRalpha and beta were localized to

myonuclei and blood vessels. GCRbeta expression levels were unaltered in muscle tissues from patients who received GC treatment prior to biopsy. In DM, increased GCRbeta expression was observed on perimysial arterioles. Scattered macrophages in normal tissues were GCRalpha and beta negative. Only few perimysial inflammatory cells in DM expressed GCRalpha and beta. In the endomysial infiltrates of PM and sIBM, a subset of macrophages and CD4+ T-cells was GCRalpha and beta positive. In sIBM, the fraction of GCRbeta positive inflammatory cells was slightly increased. Conclusions: We found GCRbeta on the endothelium of controls and IIM, and expression was upregulated in the affected endothelium of DM. Only minor differences in GCRbeta expression were observed between inflammatory cells in DM/PM and sIBM. We therefore conclude that aberrant GCRalpha/beta ratios are unlikely to be a major cause of the GC resistance in sIBM.

O79

Long-term effects of mexiletine on myotonia and on the cardiac conduction system of patients with myotonic dystrophy type 1

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Background: The anti-myotonic effect of mexiletine has been previously reported in myotonic dystrophy type1 (DM1). However, the tolerability in the long term and its effects on the cardiac conduction system remain to be clarified.

Aims: To determine the long-term effects on myotonia and the potential development of exacerbation of cardiac arrhythmias of patients with DM1 treated with mexiletine.

Methods: 48 patients with moderately-severe DM1 (CTG range 500–700 repeats; mean age 42.9 ± 12.9, mean disease duration 19 ± 11.5 years) were subjected to quantitation of myotonia by self-assessment scales and timed functional tests; muscle strength testing using the 5-point MRC scale; standard 12-lead EKG, 24-hour EKG recordings and 2D echocardiograms at baseline and after a mean follow-up of 3.5 years. 29 patients with DM1 were treated with mexiletine 200 mg tid. The remaining were not treated because either myotonia was minimal (n = 14) or there was no compliance to treatment in general (n = 5). PR interval and QRS duration, longest RR interval, percentage of supraventricular and ventricular beats were considered at baseline and on follow-up and compared between the treated and untreated groups.

Results: Improvement of myotonia was recorded in the scales and the functional tests in 24 of 29 patients treated with mexiletine. Muscle strength by MRC worsened less in the treated group compared to the untreated group (respectively from 138.6 ± 6.7 to 138.4 ± 6.2 and from 137.9 ± 4.7 to 132.6 ± 6.3, $p < 0.05$). A significant trend of progression in the PR and QRSD interval was observed in both groups when comparing initial and follow-up assessments (respectively from 182.08 ± 18.92 to 194.4 ± 16.2 and from 179.7 ± 14.3 to 185.6 ± 12.4, $p = 0.05$). No significant differences in the above cardiac parameters were observed at baseline and on follow-up in patients with DM1 treated with mexiletine compared to those who were untreated. Only the percentage of ventricular ectopic beats was significantly lower in the treated compared to the untreated group (respectively from 399.1 % ± 1463 to 117.6 % ± 295.7 and from 40 % ± 68.8 to 160.4 % ± 393.4, $p < 0.05$). Three patients with DM1 stopped treatment because of gastrointestinal symptoms, two because of symptomatic hypotension.

Conclusions: Mexiletine not only improves myotonia in DM1 but our data also suggest a parallel improvement of muscle strength. In addition, it appears to be a safe and well-tolerated drug even in patients with DM1.

Session 12

Muscle disorders 2

O80

Dysferlinopathies: muscle annexin A1 and A2 expression levels correlate with clinical phenotype in a large group of genetically diagnosed patients

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Mutations in the DYSF gene underlie two main distinct muscle diseases: Limb Girdle Muscular Dystrophy (LGMD) 2B and Miyoshi myopathy (MM). Dysferlin is involved in muscle membrane-repair and is thought to interact with other dysferlin molecules and annexins A1 and A2 at the sarcolemma. Here we performed genotype/phenotype correlations in a large cohort of dysferlinopathic patients and explored the possible role of annexins as modifier factors in LGMD-2B and MM. In particular, clinical examinations, expression of sarcolemmal proteins and genetic analysis were performed on 27 dysferlinopathic subjects. Expression of A1 and A2 annexins was investigated in LGMD-2B/MM subjects and in patients with other muscle disorders. We identified a total of 24 different DYSF mutations, 10 of them being novel. We observed no clear correlation between mutation type and clinical disease presentation, but MM patients were found to display muscle symptoms significantly earlier in life than LGMD subjects. Remarkably, dysferlinopathic patients and subjects suffering from other muscular disorders were found to express higher levels of both A1 and A2 annexin compared to controls; a significant correlation was observed between annexin expression levels and clinical severity scores. Also, annexin amounts paralleled the degree of muscle histopathologic changes.

In conclusion, our data indicate that the pathogenesis of different inherited and acquired muscle disorders, characterized by muscle degeneration and regeneration, involves over-expression of annexin A1 and A2, probably because these proteins actively participate in the plasmalemma repair process. The positive correlation between annexin A1 and A2 and clinical severity as well as muscle histopathology suggests that their level may be a prognostic indicator of disease. These observations also indicate that annexins A1 and A2 cannot be regarded as good candidates as modifier factors in dysferlinopathies

O81

About the physiopathological mechanism of statin myopathy: evidence of a diffuse reduction of CoQ10 levels in skeletal muscle

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The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, i. e. statins, are the most effective drugs for prevention and treatment of hypercholesterolaemia and coronary heart disease. They are generally well tolerated but recurrent side effects as rhabdomyolysis, myopathy and polyneuropathy have been described. Little is known about the mechanism of muscle damage but, in these patients, only anecdotal descriptions of muscle biopsies have been reported. Recently Rundek et al. (Arch, Neurol., 2004) found significantly decreased serum levels of coenzyme Q10 (CoQ10-ubiquinone) in asymptomatic subjects treated with atorvastatin, supporting the hypothesis of an inhibitory effect on CoQ10 synthesis. In the last two years, we have selected 32 patients (17F, 15M, aged from 45 to 76 years) complaining of muscle aches, cramps, proximal weakness and/or hyperCKemia after therapy with different statins (simvastatin, pravastatin, atorvastatin, cerivastatin). Their family history was unremarkable. All patients were studied with a complete workout including clinical, laboratory, electrophysiological and skeletal muscle morphological studies. Muscle biopsy revealed from minimal to marked changes in 28 patients; mitochondrial alterations were present in 18 patients (COX negative fibers in 9). Biochemical analysis of mitochondrial respiratory chain enzymes were within the normal range of controls. Southern blot analysis did not reveal any mitochondrial DNA rearrangement. Skeletal muscle CoQ10 levels were markedly decreased in 28 out of 32 patients ranging from 15 to 4 mg/g muscle tissue (control values 25 ± 4.5). Our data showed that: 1) patients treated with statins had a remarkable decrease in muscle CoQ10 concentration, lasting months after drug withdrawal; 2) statins in-

hibitory effect on CoQ10 synthesis seems to have a major role in determining statin-related muscle damage.

O82

A novel mutation in Lamin A/C gene in a Portuguese family with limb-girdle muscular dystrophy and cardiac dysrhythmia

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Background: Lamin A/C gene (LMNA/C) on chromosome 1q11-q23 encodes A-type lamin proteins. Mutations in LMNA/C are associated with several distinct pathologic conditions. One of these laminopathies is limb-girdle muscular dystrophy type 1B (LGMD1B).

Methods: We describe a Portuguese family with 12 members affected, 8 of whom are not alive. Sudden death was the cause of death in half of these patients. The 4 members alive are followed in our institution presenting a LGMD1B phenotype. The proband, a 54 year-old woman, her brother and a cousin had complaints of longstanding lower-limbs proximal muscle weakness associated with documented cardiac dysrhythmia leading to pacemaker implant and later dilated cardiomyopathy. The proband's daughter, aged 25, had complaints of mild lower-limb proximal muscle weakness and repetitive episodes of syncope without electrophysiological or structural myocardial abnormalities. All had slight elevated muscular enzymes. EMG showed a myopathic pattern without peripheral nerve involvement. Muscle biopsy had unspecific myopathic changes. Immunohistological studies were considered abnormal for lamins.

Results: Linkage analysis was initially performed for calpain-3, dysferlin, fukutin-related protein, lamin A/C and teletonin on the 4 family members. This led to the exclusion of all but LMNA/C as a candidate gene. The all LMNA/C gene was sequenced on both strands in the proband. Three alterations were detected in heterozygosity: 80C > T (exon 1), c.612G > A (exon 3) and c.1698C > T (exon 10), the first two undocumented and the latter a known polymorphism. To evaluate the causal status of these newly identified alterations, co-segregation analysis was performed showing that only c.80C > T segregated with the disorder. The causative nature of this mutation was further corroborated by population screening in 133 unrelated control individuals, bioinformatic and phylogenetic analysis.

Conclusions: These results taken together (co-segregation with the disorder, a frequency < 0.3 % and phylogenetic conservation of the threonine residue at position 27) provide strong evidence that this predicted threonine to isoleucine change (hydrophobic to polar), in the NH2-terminal region of lamin A/C, underlies the observed phenotype (autosomal dominant muscular dystrophy with LGMD1B phenotype). In this group of patients screening for carrier members should be performed since they have a four-fold higher probability of sudden death due to tachyarrhythmias.

O83

Myoblasts from affected and unaffected muscles of facioscapulohumeral muscular dystrophy patients display differences in morphology, proliferation and in vitro differentiation ability

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Objective: To assess the biological properties of myogenic cells prepared from unaffected muscles of facioscapulohumeral muscular dystrophy (FSHD) patients and compare them with that of FSHD affected muscles and matched control myoblasts in the perspective of an autologous myoblasts transfer clinical trial.

Background: Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease linked to a deletion within tandem array of repeats termed D4Z4 located on chromosome 4q. It is characterized by a typical regional distribution, featuring composed pattern of affected and unaffected muscles. No treatment is currently available for this disease whose physiopathological mechanism is still unknown. Autologous myoblast transfer from unaffected to affected territories could be considered in view of increasing the regenerating ability of affected muscles.

Methods: We produced 1) myoblasts from unaffected vastus lateralis muscle of 5 FSHD patients and myoblasts from the same muscles of 10 healthy controls; 2) myoblasts from affected shoulder girdle muscles of 4 FSHD patients and myoblasts from the same muscles of 4 healthy controls.

We evaluated in each FSHD cell culture at different time points: morphology, proliferation ability (proliferation rate, doubling time), purity of the cell culture (percentage of CD56 and desmin-positive cells), cell viability, telomere length and in vitro differentiation (myoblasts fusion index, expression of myosin heavy chain isoforms).

Results: Myoblasts prepared from unaffected muscles of FSHD patients presented no differences in morphology, proliferation ability and in vitro differentiation when compared to matched controls while myoblasts from affected muscles displayed several alterations. In particular, we could observe in these cells the appearance of "vacuolar-necrotic phenotype", reduced proliferation ability with a significant increase on cell doubling time and an impaired differentiation with an important reduction in myoblast fusion index.

Conclusion: In contrast with myoblasts from affected FSHD muscle, myoblasts from unaffected territories presented no alteration in morphology, proliferation and differentiation properties when compared to controls. These findings could open the possibility of a future clinical trial on autologous myoblast transfer for FSHD patients.

O84

Fatal R631C mutation is also present in the adult form of CPTII deficiency

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CPTII deficiency is associated with three major phenotypes: a myopathic form with juvenile-adult onset, a severe infantile form with hepatic, muscular and cardiac involvement and a fatal neonatal presentation. A great number of CPT II gene mutations have been described; so far, R631C mutation has been reported only in fatal cases. We describe herein 3 families where 4 individuals manifested the adult form with an onset ranging from 8 to 30 years. We have also examined other 9 members some of whom with minor muscular symptoms. Muscle biopsy of probands showed a mild lipid storage in two cases and was normal in one. Biochemical studies revealed a marked decrease of CPTII activity in muscle (Isotope reaction respectively 72.3, 160, 74 and 115 pmol/mg proteins; n. v. 570 ± 160 – Forward reaction respectively 118, 210, 380 and 143 pmol/mg proteins; n. v. 430 ± 180). Genetic analysis of CPTII gene revealed the presence of a homozygous R631C mutation in all four patients. Three patients expressed heterozygously the same mutation. Our data suggest that the R631C mutation is not exclusively detected in the fatal form but it may be present in a wider spectrum of CPT II phenotypes.

Session 13

Cerebrovascular disease 3

O85

Fluid-attenuated inversion recovery distal intra-arterial signal: an independent predictor of poor outcome in acute cerebral ischaemia

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Background: Although diffusion-weighted magnetic resonance (MR) imaging (DWI) and perfusion-weighted imaging are the most sensitive at the acute stage, Fluid-attenuated Inversion Recovery (FLAIR) sequences are necessary to exclude non-vascular lesions. They can also reveal intra-arterial abnormalities. The aim of the study was to evaluate the prognostic value of FLAIR abnormalities at the acute stage of cerebral ischaemia.

Methods: We studied 30 patients within 12 hours of onset of acute cerebral ischaemia with FLAIR sequences, DWI and three-dimentional (3D) time-of flight (TOF) MR. None of them received thrombolytics. The severity was evaluated by the National Institute of Health (NIH) scale at admission, and outcome at month-1 by the modified Rankin Scale (mRS). A neuroradiologist evaluated proximal and distal intra-arterial abnormalities on FLAIR sequences at admission.

Results: Of 8 patients included within 3 hours, 2 had proximal and 5 had distal intraarterial abnormalities; of 12 patients included between 3 and 6 hours, 2 had proximal and 6 had distal arterial abnormalities; of 10 patients included between 6 and 12 hours, 5 had proximal and 5 had distal arterial abnormalities. The bivariate analysis showed that DWI abnormalities ($p = 0.052$), proximal ($p = 0.001$) and distal ($p = 0.007$) occlusion on 3D TOF MR sequence and distal artery abnormalities on FLAIR ($p < 0.001$) se-

quences were associated with a bad outcome (mRS 3–6) at month-1. In logistic regression, distal intra-arterial abnormalities on FLAIR sequences were independent predictors of poor outcome (p = 0.041).

Conclusion: FLAIR distal intra-arterial abnormalities found within 12 hours of onset of acute cerebral ischaemia predict a worse short term outcome. Those patients may be candidates for a more aggressive management.

O86

Insular involvement is associated with QT-prolongation on ECG: ECG-abnormalities in acute stroke patients

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Background: Aim was to analyse electrocardiographic (ECG)-abnormalities among acute stroke patients in their relation to insular involvement, stroke severity, stroke type and cardiovascular risk factors.

Methods: Standard 12-lead-ECGs, blood samples, body temperature and blood pressure on admission were analysed in 122 consecutive patients with acute stroke. Stroke severity, stroke size and location, and cardiovascular risk factors were documented.

Results: Eighty four (69 %) patients had ECG-abnormalities on admission, most frequently ST-changes in 34 %, QT-prolongation in 31 % and atrial fibrillation in 27 % of the patients.

The frequencies of ECG-abnormalities did not differ between patients with and without coronary artery disease, with and without other cardiac comorbidity, with ischemic and hemorrhagic, and left- and right-sided strokes.

The insula was affected in 39 (32 %) patients, 31 (79 %) of them had ECG-abnormalities. Patients with insular lesion more often had a poorer functional score on admission (p = 0.000), a larger infarct size (p = 0.000) and atrial fibrillation (p = 0.028) than patients without insular lesion. Small insular infarcts were more often associated with QT-prolongation than small infarcts without insular involvement (p = 0.040). Patients with QT-prolongation more often had multiple ECG-abnormalities (p = 0.001) and a higher mean systolic and diastolic blood pressure than patients without QT-prolongation.

Conclusions: ECG-abnormalities are frequent in acute stroke patients. As QT-prolongation frequently is associated with insular lesions, continuous ECG monitoring should be mandatory in acute stroke, including patients without a history of cardiac disease. Assessment of the QT-interval should be a standard procedure in acute stroke therapy.

O87

Long-term patency of internal carotid artery after thrombendarterectomy and progression of contralateral internal carotid artery stenosis

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Objective: To evaluate (1) long-term patency of carotid endarterectomy (CEA) up to 20 years, (2) occurrence/progression of contralateral internal carotid artery (ICA) stenosis and (3) occurrence of cerebrovascular events ipsi-/contralateral to the operated ICA.

Method: 436 patients with symptomatic or asymptomatic moderate to severe (> 50 %) ICA stenosis underwent CEA between 1970 and 2002. ICA stenosis was measured by preoperative Doppler and color coded duplex sonography. All patients underwent postoperative neurosonography at 1 and 12 months respectively and annually thereafter. Recurrent stenosis > 50 % of the ipsilateral ICA (peak frequency > 4 kHz), occurrence or progression of stenosis of the contralateral ICA, and new cerebrovascular events were recorded. Survival analysis was performed with the Kaplan-Meier life table method.

Results: Restenosis of the operated ICA occurred in 43.9 % of patients in 17 years. Occurrence or progression to contralateral ICA stenosis was 58.2 % of patients for the same time period. The annual rate of restenosis of the contralateral ICA increased to 3–10.7 % 5 years after intervention, whereas the annual rate of restenosis of the operated ICA remained at 0–1.6 % during the same time period.

Cerebrovascular events occurred in 20 % of patients without progressive ipsi/contralateral stenosis, during a follow-up of 10 years. However, in those with progressive ipsi-/contralateral stenosis, 40 % of patients showed cerebrovascular events.

Conclusion: Very long term follow up (17 years) shows that restenosis after CEA is frequent; progression of contralateral stenosis is even more frequent. Progression of contralateral stenosis occurs more often > 5 years after CEA than before. Furthermore risk of cerebrovascular events is higher in patients with ipsi-/contralateral (re)stenosis than in patients without. Frequency of neurosonological follow up should be tailored to the observed time course of (re)stenosis.

O88

Hyperglycaemia in acute stroke. To be or not to be diabetic. Therapeutic implications

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Introduction: Several studies have found the deleterious effect of hyperglycemia in acute stroke, and that this effect is higher in non diabetic patients. However, a recent interim analysis of GLIA study has pointed out that up to 82 % of acute stroke patients with glucose levels higher than 150 mg/dl and no previous history of diabetes are not-known diabetic patients (glycosilated haemoglobin > 6 %). Our goal is to analyse the possible influence of previous history of diabetes in the corrective treatment of hyperglycemia in acute stroke in-patients.

Methods: GLIA study is a multicentre, prospective study to determine the glucose level associated to poor outcome adjusting for other known prognostic variables. Acute IS patients (< 24 h) were included and capillary glucose, blood pressure, body temperature and Canadian Stroke Scale (CSS) were determined each 8 h within the first 48 h. Outcome at 3 months was evaluated by means of CSS and Modified Rankin Scale. Interim analysis from GLIA Study has been developed to evaluate the applied therapies to correct hyperglycemia and the possible existence of differences regarding the previous history of diabetes.

Results: 270 acute stroke in-patients. 39.6 % had capillary glucose levels > 150 mg/dl within the first 48 h, 43.9 % of whom had no previous history of diabetes. Hyperglycemia corrective treatment (insulin or antidiabetic drugs) was used in 91.7 % of known diabetic patients but only in 55.3 % of those with no history of diabetes, but up to 50 % of them were indeed not-known diabetic patients. To have no previous history of diabetes was an independent predictor factor to not receiving hyperglycemia corrective treatment (OR 8.8 (95 % CI 3.0–26.1).

Conclusions: Acute stroke patients without previous history of diabetes have eight times higher risk not to receive any corrective treatment for hyperglycaemia although in a high percentage they are true diabetic patients. Prognostic implications of this fact should be analysed in further prospective studies.

O89

Blood pressure evolution after acute ischaemic stroke in patients with and without sleep apnoea

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Background: Sleep apnea (SA) is an independent risk factor for arterial hypertension and is present in 50–70 % of patients with acute ischemic stroke. The effect of SA on blood pressure (BP) and stroke outcome in the acute phase of stroke are poorly known.

Methods: We studied 41 consecutive patients admitted within 96 hours after onset of ischemic stroke. Stroke severity was determined by the NIH Stroke scale (NIHSS). Sleep breathing was assessed by an ambulatory device the first night after admission. SA was defined by an apnea-hypopnea-index (AHI) > 10, and moderate-severe SA (MSSA) by an AHI > 30. Monitoring of BP was performed by a portable device during the first 36 hours after admission. A non-dipping status was defined by a ratio > 0.9 of [(systolic BP during night 1 + 2)/2]/systolic BP during day 2. Stroke outcome was estimated by the modified Rankin Disability Scale (mRS) at hospital discharge.

Results: SA was found in 28 (68 %), and MSSA in 11 (27 %) of 41 patients. Patients with MSSA had higher systolic and diastolic BP values during night 1 (p = 0.001), day 2 (p = 0.002) and night 2 (p = 0.020) than patients without SA. There were no significant differences between MSSA and non-SA patients in stroke severity and outcome. A non-dipping status was found in 26 (63 %) of 41 patients. Non-dippers had a similar AHI but higher NIHSS (p = 0.004) and mRS (p = 0.008) than dippers.

Conclusions: MSSA is associated with higher 24h BP values but not with stroke severity or stroke outcome. Conversely, a non-dipping status is linked with a more severe stroke and a worse stroke outcome but not with MSSA. These data suggest different pathophysiological and clinical implications of circadian and nocturnal BP values in the acute phase of stroke.

O90
Old patients in acute stroke unit: good functional benefit at 2 months
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Background: Age is frequently an entering criterion to acute stroke units. Since functional benefit is one of the goals for these units, we compared the benefit between younger and older patients that were admitted to our stroke unit, in order to establish entering criteria based on the functional benefit of our sample.

Methods: We studied 247 consecutive patients that entered our stroke unit during the first 10 months of its functioning (144 male, 58.3 %). We defined old patients as patients older than the median age (> than 68 years). We defined functional benefit when a cut-off of at least 4 points in the Barthel revised scale was obtained at 2 months. All patients were studied according to a protocol that includes CT or diffusion MRI, cervical Triplex scan and transcraneal Doppler, ECG and transthoracic echocardiography. Selected patients were studied with transesophagic echocardiography. We considered ischaemic stroke aetiology according to TOAST criteria and stroke type according to Oxfordshire criteria. Statistical methods included logistic regression analysis.

Results: From 247 patients, 170 (69.1 %) had an ischaemic stroke, 25 a cerebral haemorrhage (10.2 %), 35 a TIA and 2 a venous thrombosis. The occurrence of functional benefit was associated with older age ($p = 0.0130$; O. R.: 2.25; 95 % CI: 1.19–4.26) and negatively with previous stroke ($p = 0.05$; O. R.: 0.54; 95 % CI: 0.54–0.99).

No relation was found between functional benefit and stroke type or aetiology except for presumed cardioembolic aetiology ($p = 0.0438$; O. R.: 2.07; 95 % CI: 1.02–4.21).

Discussion: In patients referred to our stroke unit, older patients had a better functional benefit than younger ones. Previous stroke was a negative factor in achieving functional benefit and presumed cardioembolic aetiology correlated with better outcome. Since young age is frequently part of entering criteria to stroke units, our data suggest that other criteria like cardioembolism might be considered a positive criterion for admission of old patients to acute stroke units.

Session 14

Cerebrovascular disease 4

O91
Neurovascular coupling in autonomic failure: a functional transcranial Doppler study
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Background: Neurovascular coupling (NC) adapts cerebral blood flow in accordance to cortical activity. In order to investigate the influence of autonomic failure (AF) on NC, we studied patients with familial amyloidotic polyneuropathy, a hereditary disease with severe AF.

Methods: In the sitting position a visual functional transcranial Doppler (fTCD) test was performed and the evoked flow responses in the posterior cerebral artery of 12 patients were compared with age-matched controls. Using a control system approach the dynamic features of the flow response were evaluated with the parameters gain, rate time, attenuation and natural frequency. To exclude an orthostatic influence in patients, resting flow velocities, mean blood pressure and heart rate were measured in supine and sitting positions.

Results: Resting flow velocities differed neither between the orthostatic conditions nor in relation to the control group. In the sitting position blood pressure decreased in patients from 84 ± 14 mmHg to 64 ± 25 mmHg ($p = 0.06$) and heart rate increased by 14 ± 16 beats per minute ($p < 0.05$). The parameters natural frequency (0.18 ± 0.07 l/s vs. 0.23 ± 0.5 l/s; $p < 0.05$) and rate time (2.9 ± 1.8 s vs. 4.8 ± 2.6 s; $p < 0.05$) were significantly lower in patients than in controls whereas the flow velocity levels between rest and stimulation did not differ between the groups (14.9 ± 7 % vs. 16.2 ± 6 %; p = n. s.).

Conclusion: Whereas static neurovascular coupling remained unaffected, the dynamic regulation of adequate blood flow seems to be affected in patients with autonomic dysfunction as estimated from fTCD parameters.

O92
Effects of chronic anticoagulation therapy on stroke outcome in atrial fibrillation patients
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Atrial fibrillation (AF) is a common cardiac arrhythmia met in clinical practice. It often leads to hemostatic and embolic complications. Recent data suggest anticoagulation may suppress thrombotic activity and decrease hypercoagulation state which may reduce not only the frequency but also severity of ischemic stroke (IS). A retrospective analysis was performed to assess the impact of pre-stroke anticoagulation on stroke outcome in patients with chronic atrial fibrillation.

Method: retrospective analysis of 100 consecutive, chronic atrial fibrillation patients admitted to Neurological Department within 12 hours after IS onset. The severity of stroke was established after 2 weeks according to the level of dependence measured in modified Rankin Scale (mRS). Level of anticoagulation was classified on admission according to INR values as therapeutical (INR ≥ 2.0), subtherapeutical (INR 1.5–1.9) or non therapeutical (INR 1.0–1.4). Patients receiving antiplatelet drugs were analysed along with those obtaining nontherapeutical INR.

Results: 32 % of patients received chronic oral anticoagulation, 5 % of them obtained nontherapeutic INR, 62 % subtherapeutic and 33 % therapeutic INR on admission. After 2 weeks of observation 36 % of patients were functionally nondependent (mRS ≤ 2), 44 % were regarded as dependent (mRS ≥ 3), 22 % died. The correlation between the intensity of anticoagulation and severity of stroke was noticed (r 0.32, $p = 0.0007$). Patients with subtherapeutic and nontherapeutic INR were more likely to be dependent and had higher in-hospital mortality (OR 2.89; 95 % CI 1.25–6.67, $p = 0.01$) comparing with subjects with therapeutic INR. Odds ratio for dependence and death was higher in the group of nontherapeutic (OR 2.55, 95 % CI 1.07–6.07, $p = 0.03$) than subtherapeutic INR (OR 1.11, 95 % CI 0.12–9.67, $p = 0.9$). In the model of multivariate regression including stroke risk factors and clinical data only level of anticoagulation was found to be related to course of stroke in the studied group ($p < 0.01$).

Conclusion: Chronic oral anticoagulation in patients with atrial fibrillation reduces not only risk of ischemic stroke but may influence the course of acute stroke. Subjects obtaining therapeutic anticoagulation level in the period preceding stroke onset have more favourable stroke outcome comparing to patients with sub- or nontherapeutic INR.

O93
Multiple motor areas of the contralesional hemisphere contribute to recovered hand motor function after stroke: a study with fMRI-guided navigated transcranial magnetic stimulation
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The neural basis of functional recovery after stroke is incompletely understood. Imaging techniques like functional magnetic resonance imaging (fMRI) document enhanced activity in multiple motor areas of the damaged (DAM-H) and contralesional (intact) hemisphere (CON-H). However, the functional relevance of these activity increases has been questioned. The behavioral relevance of regional activation can be tested by temporary inactivation of target areas and subsequent analysis of the induced behavioral deficits, for example with repetitive transcranial magnetic stimulation (TMS) (Gerloff et al. Brain 1997; 120: 1587–602). This approach to interfere with behavioral function has also been referred to as "jamming". Studies addressing the functional relevance of areas with increased fMRI-based activity in stroke patients are scarse and have, in relation to the CON-H, only pointed to a role of the premotor cortex (PMC) (eg, Johansen-Berg et al. Proc Natl Acad Sci USA 2002; 99: 14518–23). In these studies, TMS single pulses were used to interfere with simple reaction time tasks. The aim of the present study was to test whether multiple motor areas of the CON-H are functionally relevant for complex sequential movements of the recovered hand. Interfering bursts of high-frequency repetitive TMS ("jamming") were applied to PMC, primary motor cortex (M1) and the superior parietal lobule (SPL) of the CON-H while patients ($n = 7$) executed a previously learned finger movement sequence with the recovered hand (ipsilateral to the TMS site). TMS was stereotactically navigated (Brainsight Frameless) on the basis of 3D-reconstructed individual 3T-fMRI data. Control conditions included sham, peripheral, and aversive stimulation. Compared with age-matched controls, a significant increase in timing errors was found in stroke patients with stimula-

tion of PMC (maximal effect), SPL and M1 ($p < 0.05$, patients vs. controls). No differences between groups were present in the control conditions. In addition, stimulation of SPL induced a higher number of accuracy errors in patients ($p < 0.05$, compared with controls). These data provide first evidence for a functionally relevant contribution not only of PMC but also of M1 and SPL of the contralesional hemisphere, and, thus, start bridging the gap between imaging and TMS interference studies. The present results argue for a functionally relevant, beneficial role of the CON-H for effective recovery after capsular stroke.

O94

Magnetisation transfer imaging of normal appearing brain tissue and white matter hyperintensities in the elderly

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Background: White matter hyperintensities (WMH) are frequently observed in the ageing brain and have been associated with microangiopathy. The severity of tissue damage which is associated with these abnormalities and possible distant effects cannot be adequately determined by conventional magnetic resonance imaging (MRI). We therefore have performed a regional magnetisation transfer ratio (MTR) study to assess WMH changes and to investigate the impact of ageing and WMH on normal appearing brain tissue (NABT).

Methods: Magnetization transfer imaging was performed in 198 neurologically asymptomatic participants of the Austrian Stroke Prevention Study (mean age 70, age range 62–87 years) using a spoiled 3D gradient echo sequence on a 1.5T scanner. MTR measurements were done in WMH and in NABT using predefined templates. Fluid attenuated inversion recovery MRI was used to delineate lesions and to grade their severity.

Results: The individuals' age showed a modest but significant effect on the MTR of NABT ($p < 0.01$). The highest age dependency was found for the frontal and parieto-occipital cortex, where we observed a relative annual MTR decrease of 0.16 % and 0.21 %, respectively ($p < 0.01$). The MTR of WMH was always significantly lower than that of normal appearing white matter (NAWM) with an overall relative reduction of about 10 % and decreased significantly with increasing scores of WMH severity ($p = 0.02$) and WMH volume ($r = -0.24$, $p = 0.0016$). MTR was not different between subjects with very few and extensive WMH. The WMH volume was associated with NAWM MTR in the frontal lobe and corpus callosum but not in other regions of the brain.

Discussion: The tissue damage associated with WMH is quantifiable with magnetization transfer imaging. It is relatively mild and increases with lesion size. We could not observe distant effect of the WMH except for the frontal lobe and the corpus callosum which supports the concept of a focal origin of WMH. NABT seems to change with normal aging per se.

O95

Evidence for aetiologic heterogeneity of white matter hyperintensities in the elderly: subanalysis of the Austrian Stroke Prevention Study using lesion probability maps

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Background: White matter hyperintensities (WMH) are common on brain MRI of clinically asymptomatic, elderly individuals. Their appearance can be characterized as either punctate, early confluent or confluent. While this increase in extension is frequently seen as evidence for a continuum of changes associated with a common mechanism, histological data and clinical follow-up suggest fundamental differences. We hypothesise this is also reflected by distinguishable spatial distributions.

Methods: We tested this hypothesis by exploring the distributions of punctuate and confluent lesions using lesion probability maps (LPM) generated from MRI scans of 189 participants (mean age 60.8 ± 6.2 yrs) in the Austrian Stroke Prevention Study. We dichotomised WMH according to the classification by Fazekas et al. [punctate ($n = 143$) vs. early confluent and confluent ($n = 33$)] to run voxel-based t-tests using permutation-based nonparametric inference. To test alternative hypotheses, we created similar LPM for age and arterial hypertension.

Results: We observed significant differences in the spatial distribution of lesions for the two WMH groups ($p < 0.01$). Punctate lesions were more diffusely distributed throughout the cerebral white matter (peak probability 5 %) relative to confluent lesions (peak probability 45 %). Confluent lesions had greatest likelihood of being found in perfusion "watershed" regions. These differences in distribution could not be explained by differences in age or hypertension only, as both greater age and the diagnosis of hypertension were associated with WMH abutting the occipital horns.

Conclusions: Punctate and confluent WMH of elderly individuals show a different spatial distribution which strongly supports etiologic heterogeneity. Preferential localization of the more confluent WMH within arterial watershed areas suggests a prominent ischemic component in their development.

O96

Dural sinus thrombosis presenting as isolated intracranial hypertension: always benign?

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on behalf of the ISCVT collaborators

Background: Isolated intracranial hypertension (IIH) is a distinct clinical presentation of cerebral venous thrombosis (CVT). There is few information about the clinical course and long-term outcome of CVT presenting with IIH.

Aim: to describe the clinical course and outcome of patients presenting with IIH from the International Study of Cerebral and Dural Sinus Thrombosis (ISCVT).

Methods: ISCVT is a multinational, prospective, observational study that included 624 patients with CVT. IIH was defined as any combination of headache, vomiting and papilloedema, with/without visual loss or VI nerve paresis, without other neurological symptoms or signs. Demographic, clinical features, mode of onset, sinus occlusion, cerebral lesions on admission CT/MR, risk factors, and the long-term outcome were analysed in patients with IIH, and compared with patients with other presentations of CVT (chi-square test, with Yates correction when necessary; Student's t test).

Results: 143 (23 %) patients had IIH, mainly with subacute (59 %) or acute (30 %) onset. 26 patients (18 %) had asymptomatic cerebral lesions on CT or MR scans. Compared to other clinical presentations, isolated unilateral lateral sinus thrombosis was more frequent in patients with IIH (36 % vs. 19 %, $p < 0.001$), the superior sagittal sinus (55 % vs. 64 %, $p = 0.033$) and deep cerebral venous system (5 % vs. 13 %, $p = 0.008$) were less frequently thrombosed. 122 (85 %) patients were anticoagulated with heparin. Raised intracranial pressure was treated by lumbar punctions (8 patients, 5.6 %), or a shunt (5 patients, 3.5 %). Neurological worsening occurred in 22 (15 %) patients: depressed consciousness ($n = 9$), mental state disturbance ($n = 5$), focal deficit ($n = 11$), seizure ($n = 4$), and visual loss ($n = 6$). The outcome of patients with IIH was significantly better than that of the other patients. All patients worsening during the acute phase made a complete recovery. At the last follow-up (median time 14 months), 129 (90 %) patients had recovered completely, 4 (2.8 %) were dependent, and 6 had died. Visual complaints (13 % vs. 5 %, $p = 0.003$) and severe headaches (21 % vs. 13 %, $p = 0.019$) during follow-up were more frequent among patients with the IIH syndrome. Causes of death were: malignancy [3] myocardial infarct, gastro intestinal bleeding, and unknown [1].

Conclusions: Although most patients with CVT who present with IIH have an excellent outcome, clinical deterioration is not rare, and permanent visual impairment may occur.

Session 15

Extrapyramidal disorders 1

O97

Long-term follow-up (24 months) of quetiapine-treated psychotic Parkinson's disease patients

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Background: Parkinson's Disease (PD) patients treated long-term with levodopa (LD) frequently develop psychosis which is difficult to treat. Among the different available atypical antipsychotics in use, clozapine (CLOZ) has been shown to be the most effective drug, although its safety profile requires a close follow-up (Klein et al. Clin Neuropharmacol, 2003). Quetiapine (QTP) is the second most common drug used. However, experience with long-term follow-up is limited.

Objectives: The objective of this study was to evaluate the long-term outcome of QTP use for psychotic PD patients.

Patients and Methods: Thirty-five psychotic PD patients (pts) (mean age 76.1 + 5.9 y.; mean disease duration 10.3 + 5.3 y., 10 of them demented) were followed up for 24 months. Pts included 9 who only suffered from visual hallucinations and 26 who suffered from delusions or a combination of hallucinations and delusions. All pts were treated with LD (mean daily dose 685.3 + 313 mg) and some of them (n = 20) also received dopamine agonists (mean daily dose of pergolide (or its equivalent) (0.91 + 0.5 mg). QTP was given in a flexible dose (mean daily dose 116 + 74 mg). The evaluation included a clinical interview or telephone screening.

Results: After two years, 11 patients (31 %) were still receiving QTP treatment (7 with dementia). In this group, a complete resolution of symptoms was observed in 7 pts and a partial resolution in 4 pts. Treatment was stopped in 24 pts: 15 (42 %) due to lack of response (11 within the first 3 months); 3 pts due to marked improvement with resolution of symptoms; 1 due to severe somnolence; 3 pts for personal reasons; and 2 passed away. We did not find any demographic or clinical difference among patients who continued or stopped treatment. Among 15 pts with a poor outcome on QTP, 12 were switched to CLOZ (7 demented, mean dose CLOZ 27.2 + 14.3 mg) and in 3, a combination of QTP and CLOZ was used. Under CLOZ treatment a complete resolution of symptoms was observed in 9 pts and partial improvement in 3 pts. Three pts (3/35; 9 %) were resistant to both treatments.

Conclusion: Long-term treatment (24 months) with QTP shows a beneficial effect in 30 % of psychotic PD pts. In resistant QTP patients, CLOZ is undoubtedly a useful alternative.

O98

Smoking and tea consumption do not affect progression of Parkinson's disease

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Previous epidemiological studies found a negative association between cigarette smoking and coffee and/or tea drinking with the occurrence of Parkinson's disease (PD). However, it is unknown how these factors affect the rate of progression of the disease. A retrospective study was conducted among 278 consecutive PD patients. Data on smoking and coffee or tea consumption were obtained through direct or proxy interviews. Cox proportional hazards model was used to estimate whether the dependent variables affect the rate of progression of the disease. Progression of the disease, measured by the time it took to reach Hoehn & Yahr stage III, was not affected by history of smoking or tea and coffee consumption. Smoking, tea and coffee consumption had no effect on disease progression.

O99

Frequency of restrictive valvular heart disease in patients with Parkinson's disease treated with pergolide, an ergot-derived anti-parkinsonian drug: a case-control echocardiographic study

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Background and Objective: Severe restrictive valve disease is a rare cardiac complication of treatment with pergolide, an ergot-derived dopamine receptor agonist anti-Parkinsonian drug. The objective of our study was to determine the frequency of overt restrictive valve disease, as well as the relative frequency of minor valve abnormalities, in patients (pts) with Parkinson's disease treated with pergolide.

Methods: Echocardiography was performed in 36 pts with Parkinson's disease treated with pergolide (median age: 68 yr (range: 50–83 yr); 44 % men; median daily pergolide dose: 2.0 g/d, median treatment duration: 36 mo, median cumulative pergolide dose: 1643 g) and 31 age-matched pts with Parkinson's disease who were never treated with the drug (control group; age: 69 yr (43–82 yr); 58 % men). All pts had normal left ventricular size and systolic function (global and segmental). Valve morphology and function and pulmonary artery pressures were compared between the 2 groups.

Results: Restrictive valve disease with no other apparent cause was evident in 2 pts treated with pergolide (5.6 %; restricted mitral valve motion with moderately-severe mitral regurgitation in 1 pt and restricted tricuspid valve motion with moderate tricuspid regurgitation in 1 pt), versus none in the control group (p = 0.50). Overall, there were no significant differences in multiple valve parameters (frequency of valve leaflet thickening; frequency and severity of valve regurgitation (aortic, mitral, and tricuspid valves); Doppler evidence of minor degrees of aortic valve obstruction (increased trans aortic flow velocities); 2-dimensional evidence of mitral valve leaflet tethering (mitral valve tenting area and tenting distance); p values > 0.10) and no difference in pulmonary pressures between the 2 groups (35 ± 8 mm Hg in pergolide-treated pts versus 32 ± 5 mm Hg in untreated pts; p = 0.18). Moreover, there were no differences in these parameters between pts treated with higher daily doses (> 2.0 g/d) or higher cumulative doses (> 1643 g) of pergolide (p values > 0.10).

Conclusions: Restrictive valve disease is an uncommon complication of treatment with pergolide. The low frequency of minor valve abnormalities in pts without overt valve restriction and lack of a dose-response association suggests that pergolide-associated valve disease is an idiosyncratic complication of the drug.

O100

Twenty years of experience in Wilson's disease

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Background: Wilson's disease is a rare metabolic disease with different presenting features causing a considerable delay in its diagnosis and treatment.

Patients and setting: A series of Iranian patients with Wilson's disease admitted at the neurology department of Shariati University Hospital from 1984 to 2004.

Results: Forty-four patients were enrolled with the youngest being an 8-year-old girl and the oldest a 40-year-old man. The most common presenting symptom was difficulty in writing and the second was speech problem. The longest delay in definite diagnosis was one year. In 20 patients, unusual presentations such as abdominal pain, convulsion, hysteriform gait, mental retardation, psychotic features, and petechial hemorrhages caused the delay in considering the diagnosis. One patient with thrombocytopenia even underwent splenectomy before being referred to our center. Five cases died because of delay in diagnosis or unresponsiveness to treatment.

Conclusion: The timely diagnosis of Wilson's disease needs a high index of suspicion, as many patients may present with unusual signs and symptoms.

A video presentation of some interesting cases (before and after treatment) will be available at the congress session.

O101

Very late-onset Friedreich's ataxia with minimal GAA1 expansion mimicking multiple system atrophy of cerebellar type

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Background: Up to 25 % of Friedreich's ataxia (FA) patients, due to homozygous GAA repeat expansions within the FRDA gene, may be considered atypical according to classical diagnostic criteria including symptomatic onset at 40 years or older: very late-onset Friedreich's ataxia (VLOFA).

Objective: To describe the case of a VLOFA patient with a clinical picture mimicking multiple system atrophy of cerebellar type (MSA-C).

Patient, Methods and Results: This man aged 75 years suffered from progressive gait instability and falls as of age 56. Later on he also presented dysarthria, intermittent dysphagia, erectile dysfunction and urinary urgency and incontinence. Cognitive decline did not occur. Examination, which will be illustrated with video-recording, showed severe gait ataxia, only possible with support, and complete loss of postural reflexes. There was marked appendicular dysmetria with coarse terminal tremor on finger-to-nose test as the finger approached the nose. Tendon reflexes were exaggerated; plantar responses were extensor. Lower limb hypopallesthesia predominating in the feet was noted. There were saccadic intrusions during smooth pursuit and horizontal gaze-evoked nystagmus. Scoliosis, pes cavus and orthostatic hypotension were absent. Patient's parents were not consanguineous and died at an advanced age with no evidence of ataxia. His two daughters were asymptomatic, examination of the elder one aged 49 being normal. None of his 5 siblings suffered from ataxia. An initial working diagnosis of MSA-C was given. Routine laboratory investigations including vitamin E levels were normal. Electrophysiological study revealed the following findings: i) attenuation of distal sensory nerve potential amplitudes with preservation of sensory conduction velocities; ii) severe attenuation of somatosensory-evoked potentials with prolonged latencies from both tibial nerves and preservation from median nerve; iii) prolongation of central motor conduction time to upper and lower limbs; and iv) normal motor nerve conduction parameters, brainstem auditory

evoked potentials and electromyography of tibialis anterior muscle. Neither ECG nor echocardiogram showed abnormalities. MRI illustrated cerebellar and spinal cord atrophy. Molecular genetic analysis in the patient showed a homozygous expansion of 67 and 233 GAA repeats, whereas his asymptomatic daughter exhibited a heterozygous expansion of 20 and 255 GAA repeats.

Conclusion: FA with minimal GAA1 expansion may manifest with a novel VLOFA phenotype mimicking MSA-C.

O102

Sensory processing is impaired in patients with focal dystonia

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The term "Movement Disorders" is often used to subsume conditions resulting from basal ganglia (BG) pathology and it refers to the predominating motor symptoms. However, clinically unapparent sensory deficits were also found. Recent studies have revealed multimodal sensory deficits impaired in various BG disorders. We examined kinaesthesia and the habituation of the auditory startle reflex (ASR) in focal dystonia (FD) and Parkinson's disease (PD) to obtain further knowledge on the structures involved in sensorimotor processing.

Methods: The subject group consisted of patients with FD (n = 12), patients with PD (n = 11) and an age matched group of healthy controls (n = 13). Median nerve SEP did not show any evidence for a conduction deficit in FD patients. First, we analyzed kinaesthesia in an automated passive movement task (extension and flexion of the index finger between 0.2° and 4° in 45 trials of random order). Secondly, we examined the habituation of the startle reflex. 42 consecutive stimuli (110 decibel, randomized interstimulus intervals of 30–40s) were bilaterally applied, surface EMGs were recorded from the orbicularis oculi (OOM) and the sternocleidomastoid muscles (SM).

Results: a) Kinaesthesia: controls detected 82 % of 0.2° displacements, PD patients only 59 %. FD patients also revealed a kinaesthetic deficit (76 % correct responses). Differences from controls were significant (χ^2, p = 0.001 for PD, p = 0.002 for FD). Calculated thresholds for 75 % correct responses was double in FD patients (0.2°) compared to control subjects (0.1°) and three times higher in PD patients (0.3°). b) ASR: peak amplitudes and integrated muscle responses were significantly higher in PD than in controls and highest in FD (p = 0.001). Habituation was present in all groups although the extent of habituation was less marked in FD than in PD and controls.

Conclusions: The main new result of the present study is the finding of an impaired kinaesthesia in FD. Moreover, habituation of the ASR appeared to be reduced in FD, whereas startle amplitudes were significantly increased compared to controls. EMG responses and decreased habituation indicate impaired ASR circuit inhibition and could represent sensory overflow mechanisms that were also demonstrated in studies of other sensory modalities. Taken both experiments together, impaired sensory processing seems to be a common feature in BG disorders. Our results point to the potential role of the BG as a sensory analyser.

Session 16

Extrapyramidal disorders 2

O103

Impairment of kinaesthesia in Parkinson's disease and its reversal by STN-DBS

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Kinaesthesia is defined as the conscious perception of active or passive motion and direction of movements. Several studies indicated that patients with Parkinson's disease (PD) might have an altered perception of sensory signals. We addressed the question whether the basal ganglia are involved in the perception of limb position. Therefore, we sought to determine if patients with Parkinson's disease (PD) show an impaired kinaesthesia and if yes, this impairment might be at least partially reversed by STN-DBS. The paradigm consisted of a passive movement task. The subject's forearm was moved passively (~ 0.5 deg/s) while resting on a splint. A torque motor was mechanically connected to the splint, moving it at a constant speed causing either forearm flexion or extension. In the first experiment, 9 PD patients were compared with 6 patients with cerebellar degeneration (SCA6 and 8) and 11 age-matched healthy control subjects. In comparison to control subjects, PD patients but not SCA patients were significantly impaired in the ability to correctly detect displacements. A 1° forearm displacement was correctly recognized in over 75 % of trials by control subjects and SCA patients, but only in 55 % of PD patients. In the second experiment, 9 PD patients with bilateral chronic STN DBS and 7 controls were tested. Patients were tested while the stimulator was turned on and off. A 1° displacement was detected in 78 % of trials and a 2° displacement in over 91 % of trials by the control subjects. In contrast, PD patients were clearly impaired in correct detection of movement direction. During ON stimulation they showed correct responses in 74 % for 2° displacements. Deficits worsened when the DBS device was OFF with PD patients correctly detecting 2° displacements in only 60 % of trials. Thresholds for 75 % correct responses were 0.9° for controls, 2.5° for PD patients when stimulation was OFF and 2.0° when stimulation was ON. In summary, we conclude that an intact cerebro-basal-ganglia loop is essential for awareness of limb position. Moreover, our results imply that STN DBS may partially improve kinaesthesic deficits in PD.

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O104

Sensory abnormalities in unaffected relatives in familial adult onset primary torsion dystonia: an endophenotype?

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Objectives: The aim of this study was to assess the spatial discrimination threshold (SDT) in four multiplex families with adult onset primary torsion dystonia (AOPTD). The hypothesis is that abnormal SDT values in otherwise clinically unaffected family members may represent an endophenotype.

Background: There is increasing evidence of sensory abnormalities in patients with AOPTD including recent reports of structural abnormalities in the primary sensory cortex. The search for a genetic cause of AOPTD is hampered by lack of informative families due to markedly reduced penetrance of the phenotype.

Methods: SDTs were measured using Johnson-VanBoven-Phillips domes in controls and family members from four multiplex families with AOPTD.

Results: The mean SDT (± SD) of 70 normal control subjects aged 20–45 years was 1.157 mm ± 0.328; the upper limit of normal of the SDT for this age group was 1.977 mm (mean + 2.5 SD). An upper normal limit for the SDT could not be determined for control subjects more than 45 years of age because of a ceiling effect. In 54 family members aged 20–45 years abnormal SDTs were found in four of five individuals affected with AOPTD and in 12 of 49 unaffected relatives: six of 13 children, one of eleven siblings and five of 25 second degree relatives.

Conclusion: We conclude that these sensory abnormalities may represent a structural and or physiological cerebral disorder and may be a surrogate marker for the carriage of an abnormal gene. This abnormality may or may not be expressed later as AOPTD.

O105

Association of alpha-synuclein Rep1 polymorphism with risk for Parkinson's disease and influence on age at onset of Parkinson's disease

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The alpha-synuclein Rep1 promoter polymorphism, a mixed dinucleotide repeat is thought to participate in the control of gene expression and has been examined in case-control studies for a possible association with sporadic PD, but results were contradictory. Moreover, five studies examined the influence of Rep1 polymorphism on age at onset of PD with negative results.

In present study, the alpha-synuclein Rep1 polymorphism was studied in 178 sporadic PD patients (men/women ratio = 1.5; age (mean = 69, 5 years, range = 44–95 years); age at onset (mean = 63, 3, range = 30 to 88

years)) and 186 age- and gender-matched controls in an ethnic Greek population.

The promoter region of alpha-synuclein gene containing the Rep1 dinucleotide polymorphism was amplified in a PCR with forward and fluorescently tagged reverse primers Fam5'-CCT GGC ATA TTT GAT TGC AA-3' and 5'-GAC TGG CCC AAG ATT AAC CA-3'. PCR products were sized by capillary electrophoresis on an ABI 3100 analyser and analysed using Genotyper software.

The genotype distributions of Rep1 polymorphism were in Hardy-Weinberg equilibrium in controls ($p > 0.05$). Six polymorphic alleles (designated allele −2 = 263 bp, −1 = 265, 0 = 267, 1 = 269, 2 = 271, 3 = 273) according to the nomenclature previously described (Xia et al. 1996). Using a logistic regression analysis we found an association of allele 2 with risk of Parkinson's disease (PD) (adjusted odds ratio = 3.25; 95 % CI = 1.80–5.87). Survival analyses (Cox proportional hazards models) were employed to explore the influence of genotypes on age at onset of PD. Age at onset of carriers of at least one Rep1 allele 2 was earlier (3.6 years) compared to non-carriers (adjusted hazard ratio = 2.21; 95 % CI = 1.58–3.10). Kaplan-Meier analysis supported also a dosage effect of Rep1 allele 2 on age at onset.

In conclusion, we report on association of Rep1 allele 2 with increased risk for PD and for the first time we present data supportive of an influence of Rep1 polymorphism on age at onset of PD.

O106
Rapid-onset dystonia-parkinsonism: a longitudinal study of an Irish kindred
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Introduction: Rapid-onset dystonia-parkinsonism (RDP, DYT12) is an autosomal-dominant movement disorder characterized by abrupt onset of dystonia and parkinsonism with prominent bulbar and upper limb involvement, and a poor or absent response to dopamine agonists. It has previously been reported that most patients remain stable or demonstrate slight improvement years after the onset of symptoms, but there is a paucity of longitudinal studies and video material available on this rare condition. In the last 4 years 2 European kindreds have been described, and it is likely that more will be recognized in the future.

Methods: We previously published a detailed review of eight affected members from an Irish kindred. In this follow-up study performed 7 years later, we re-examined and videoed this kindred, and evaluated changes in disability status overtime.

Results: A new patient, from the third generation of this kindred, has presented recently. This 20 year old male developed abrupt onset of dystonic posturing and parkinsonism affecting his right upper limb, with minimal dysarthria and right lower limb involvement. His brother, now 24 years old, presented in 1998 with an onset over 10 days of dysarthria, dysphagia and limb dystonia. He demonstrated progression over a seven year period to a severe level of disability. He requires gastrostomy feeding, is largely wheelchair dependent and requires regular botulinum toxin injections for severe left upper limb contractures. There are 6 other affected surviving members of the kindred. Three had a gradual onset, minimal progression and relatively mild levels of disability, while three others had a rapid onset and progression to varying degrees of more severe disability. Some of those more severely affected family members have functionally improved with intensive rehabilitation.

Discussion: RDP causes limb and cranial dystonia with dysarthria and dysphagia accompanied by bradykinesia, slow gait, and postural instability. Though many patients remain stable, some may progress and may be severely disabled requiring enteral feeding and total care. This report demonstrates that there is a broad spectrum of phenotype and severity associated with RDP, with many patients demonstrating lower limb involvement. The broader spectrum of neurological abnormalities described in this study and demonstrated in the video will assist clinicians in making this diagnosis, should they encounter RDP in the future.

O107
Are Parkinson carriers specially fit to be stimulated?
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The usual clinical features of carriers of parkin mutations are early onset typical parkinsonism with a slow clinical course, good or excellent response to low doses of levodopa with frequent treatment-induced dyskinesias and the absence of dementia (Luecking et al. 2000, Khan et al. 2003). Neuropathological examination of parkin cases showed severe generalized loss of dopaminergic neurons in the substantia nigra pars compacta (Hayashi et al. 2000, van de Warremburg et al. 2001, Gouider-Khouja et al. 2003). High-frequency stimulation of the subthalamic nucleus (STN) is increasingly accepted as an adjunct therapy in Parkinson disease (PD). In order to determine the effects of high-frequency STN stimulation in early-onset parkinsonism, we have compared the follow-up after surgery (6 and 12 months) of a series of 14 patients with and 48 patients without parkin mutations.

Clinical presentation evaluated by UPDRS (I to VI), neuropsychological evaluation (Mattis, frontal scores) and daily dose of antiparkinsonian treatment at time of surgery were similar in all patients, despite the significant by lower age at onset in parkin patients (30.3 ± 11.7 years versus 37.4 ± 9.4 years, $p < 0.05$). Six and 12 months after neurosurgery, there was no difference between the UPDRS scores and neuropsychological states of the two groups of patients. Nevertheless, the levodopa-equivalent daily dose was lower in parkin carriers than in non-carriers after neurosurgery ($190 \text{ mg} \pm 211 \text{ mg}$ versus $361 \text{ mg} \pm 380 \text{ mg}$, $p < 0.05$).

These results suggest that carriers of parkin mutations are highly indicated for STN stimulations and the result of surgery may be more durable.

Session 17

Neuro-ophthalmology and vestibular disturbance

O108
Cerebellar infarction affects visual search
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In recent studies the cerebellum has been discussed to be involved not only in motor but also in cognitive functions. Particularly in frontoexecutive functions patients with cerebellar lesions showed deficits. Similar to a previous study on hemineglect patients, 3 visual search tasks (implying feature search for color and shape and conjunction search) was applied to investigate 8 patients with isolated infarction of the cerebellum compared to a group of age-matched controls. The cerebellar patients showed longer search durations, associated with a higher number of repeated saccades towards the same items. Systematic geometric search strategies were similar in both groups. Concluding, basic mechanisms of visual search are not affected by cerebellar lesions while patients' search behaviour is generally less efficient, due to redundant saccades, indicating a mild deficit of spatial working memory.

O109
Short-term efficacy of Epley's manoeuvre for benign paroxysmal positional vertigo: a randomised double-blind placebo-controlled trial
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Introduction: Benign paroxysmal positional vertigo of the posterior canal (PC-BPPV) can be effectively treated with Epley's maneuver (EM). However, the magnitude of its effect has been questioned as PC-BPPV may resolve spontaneously. So far, none of the many trials on the efficacy of EM has measured short-term efficacy as required for evaluating treatment of a spontaneously resolving disorder.

Methods: Sixty seven patients with acute unilateral BPPV of the posterior canal confirmed by Dix-Hallpike testing were randomly assigned to treatment with Epley's maneuver (n = 36) or a sham procedure consisting of an EM for the healthy side (n = 31). In the verum-group, Dix-Hallpike testing and EM was repeated until no more nystagmus and vertigo could be elicited. Patients in the EM-group received up to three maneuvers while patients in the placebo-group received a corresponding number of placebo-maneuvers. On the next day, outcome was assessed by a blinded investigator. Successful treatment was measured by the absence of both positional nystagmus and vertigo. Subsequently, all patients with a still positive Dix-Hallpike-test were treated with EM. Subjective outcome was again assessed by means of a telephone interview one day thereafter.

Results: Treatment of PC-BPPV with the EM was more effective than the sham procedure: after one day, 28 of 35 patients (80 %) in the EM-group had neither vertigo nor nystagmus on positional testing compared to three

of 31 patients (10 %) in the placebo-group ($p < 0.001$). In the EM-group 43 % of successfully treated patients had a single EM, whereas 57 % needed more than one EM.

Conclusions: This double-blind, placebo-controlled trial on the short-term outcome of EM shows that EM is highly effective in the treatment of PC-BPPV. In about half of the patients repeated EMs during one session are required for resolution.

O110

Do vestibular deficits cause somatoform disorders?

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Organic vestibular and somatoform vertigo syndromes show a high coincidence. Several studies have proposed pathogenetic models of a strong linkage between organic and somatoform vertigo. One hypothesis is that a persisting vestibular deficit causes the development of an anxiety disorder. In an ongoing interdisciplinary study 121 participants underwent structured neurological and psychosomatic diagnostic procedures. The aim of this study was to analyze the relationship between vestibular deficits and somatoform disorders.

Methods: The participants were divided among eight diagnostic groups: 1. healthy controls (HC) n = 27; 2. patients with benign paroxysmal positional vertigo (BPPV) n = 15; 3. vestibular neuritis (VN) n = 11; 4. Meniere's disease (MD) n = 6; 5. vestibular migraine (VM) n = 15; 6. anxiety n = 17; 7. depression n = 11; and 8. somatoform disorders n = 19. The neurological diagnostic procedures included electro-oculography with both rotatory and caloric testing, measurements of the subjective visual vertical (SVV), ocular torsion by fundus photography, and a neurological and neuro-otological examination. The psychosomatic diagnostic procedure comprised interviews and standardized psychometric instruments.

Results: Patients with BPPV (35.3 %) and with VN (52.2 %) had pathological values during caloric testing in both ears ($p = 0.05$). Only patients with VN showed tilts of the SVV of 4.3 degree ($p = 0.05$). Patients with MD, VM, and psychiatric disorders had normal values for vestibular testing but pathological values for psychometric measures. No correlation was found between pathological neurological and psychometric parameters.

Conclusion: No correlation was found between the results of the vestibular testing and the psychometric testing. Thus, higher anxiety scores are not caused by vestibular deficits or dysfunction as hypothesized earlier. Patients with MD and VM but without vestibular deficits showed the highest psychiatric comorbidity. Therefore the course of the vertigo syndrome and the possibility of a pre-existing psychopathological personality structure should be considered strong pathogenetic factors in the linkage between organic and psychosomatic vertigo syndromes.

O111

Anterior semicircular canal stimulation induces ocular torsion but no displacement of the subjective visual vertical

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Tonic effects in the roll plane of the vestibulo-ocular reflex such as tilts of the subjective visual vertical (SVV) and ocular torsion (OT) were thought to be caused by otolith stimulation [1, 2]. It has been shown that during galvanic stimulation of the vestibular nerve tonic OT was in 80 % of cases due to a stimulation of the semicircular canals (SCC) and in 20 % due to otolith stimulation [3].

Aim: To analyse tonic vestibular signs and perception of verticality by measurements of SVV and OT in 17 healthy subjects during the stimulation of different SCC by caloric irrigation (CI). For optimal stimulation of horizontal (hSCC) and anterior (aSCC) semicircular canals different head positions were chosen. We tested if CI of the aSCC induces tilts of the SVV and/or OT.

Methods: SVV measurement and fundus photography (FP) were performed before (baseline) and after CI of the right ear in 17 healthy volunteers (9 males, 8 females; mean age 31 years). SVV was measured binocular in a static mode, FP with a laser ophthalmoscope. CI was performed twice with cold water (13 °C) for 50 s (2 ml/s). SVV and FP were measured after CI under 4 conditions: (1) in supine position with the head tilted 30° up relative to the horizontal plane (HP) 1 minute (min) after CI, (2) in a prone position 120° from the HP 3 min after a first and a second CI, (3) condition 2 after 1 min, (4) condition 2 after 3 min. Condition 1 generated data during CI of the hSCC, conditions 2, 3, and 4, of the aSCC.

Results: No tilts or differences of SVV were found under the 4 conditions. OT was induced toward the irrigated ear during the stimulation of the aSCC ($p = 0.017$), but not of the hSCC. Significant differences were found between conditions 2 and 3 ($p = 0.02$) and between conditions 3 and 4 ($p = 0.028$). The highest OT of about 3° was seen under condition 3.

Conclusion: Stimulation of the hSCC did not induce a tilt of SVV or OT. Stimulation of the aSCC caused an OT of both eyes without perceptual tilts. This dissociation shows that an isolated OT can occur during aSCC stimulation, and tilts of SVV are caused by otolith stimulation. This agrees with a case report (4) of a patient with an isolated dysfunction of the aSCC who exhibited significant OT toward the affected ear but no SVV tilts. Measurements of OT evaluate otolith and/or vertical canal dysfunction, whereas measurement of SVV evaluates otolith function only.

References

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O112

Normal and abnormal fMRI activation patterns in the visual cortex after recovery from optic neuritis

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Optic neuritis is a common condition that causes a reversible loss of vision. Recovery to normal or near normal visual acuity is common, despite frequent persistence of conduction abnormalities, evident in prolonged visual evoked potential (VEP) latencies. It is commonly accepted that improvement of visual function is due to a peripheral nerve recovery. However, central reorganization processes at higher cortical levels may also be involved. Our aim was to assess this by comparing the patterns of fMRI activation along the visual cortical hierarchy, elicited by stimulation of the affected and normal eye. To that end, we compared the fMRI signals evoked during epochs of object images to those evoked by scrambled objects. This allows demarcation of higher object-related areas as well as primary visual areas. Activation was assessed in 8 subjects, which recovered clinically from an acute episode of unilateral optic neuritis but still had prolonged VEP latencies. In all patients, reduced activation was seen in the early retinotopic visual areas during stimulation of the affected eye (compared to the normal eye). This was in contrast to higher object related visual areas in the lateral occipital complex (LOC), in which there was no difference in the fMRI activation elicited by the two eyes. The fMRI signal difference between the two eyes decreased in magnitude with progression along the visual hierarchy. It was significant, in V1-V3, whereas in V4, the difference was negligible. Furthermore, in specific object related visual areas (inferior & lateral occipital gyri), an opposite effect was present, showing significantly higher fMRI signal in response to stimulation of the affected eye. These results may indicate a built-in robustness of the higher order object-related areas to disruption of the visual input. Alternatively, it could reflect an adaptive functional reorganization of the cortical response to an abnormal input.

O113

Retinal nerve fibre layer thickness correlates with visual dysfunction following optic neuritis

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Background: Axonal loss is the likely substrate for persistent loss of function following relapses in multiple sclerosis but direct in vivo evidence is lacking. Optic neuritis is an ideal model to study the mechanisms of persistent dysfunction since retinal nerve fibre layer (RNFL) imaging provides a non-invasive measure of axonal loss

Objective: Our hypothesis was that axonal loss of the RNFL and macula – quantified in vivo by optical coherence tomography (OCT) – is correlated with persistent visual dysfunction following optic neuritis.

Methods: We studied patients at least one year after a single unilateral attack of optic neuritis without recurrence, with a selection bias towards incomplete recovery. 25 patients and 15 controls had OCT measurement of RNFL thickness and macular volume (MV), quantitative visual testing, and electrophysiological examination with Visual Evoked Potentials (VEP) and Pattern Electroretinogram (PERG).

Results: In affected patient eyes compared to control eyes, RNFL thickness was reduced by 33 % ($P < 0.001$) and MV by 11 % ($P < 0.001$). Both

measures were also significantly reduced when compared to clinically unaffected fellow eyes. Within affected patient eyes, visual field mean deviation correlated with RNFL thickness ($r = 0.50$, $p = 0.01$) but not MV, and colour vision (Farnsworth-Munsell 100-Hue score) correlated with both RNFL thickness ($r = -0.60$ $p = 0.002$) and MV ($r = -0.60$ $p = 0.002$). VEP amplitudes which reflect optic nerve axonal integrity correlated with both OCT measures. The PERG N95 amplitude, thought to be of ganglion cell origin, correlated with RNFL thickness only. When inter-ocular differences were used to take into account background inter-subject variability in visual function and RNFL, the previous associations with visual function became stronger, with the emergence of additional correlation of OCT measures with visual acuity (RNFL: $r = -0.65$, $p < 0.001$; MV: $r = -0.47$, $p = 0.02$).

The superior and inferior RNFL quadrants (the thickest parts of the RNFL) significantly correlated with their corresponding visual field sector function. MV was correlated with the temporal RNFL, the destination of macular axons.

Conclusion: This study demonstrated functionally relevant and anatomically coherent changes in the RNFL of eyes affected by optic neuritis and supports the hypothesis that axonal loss causes persistent visual dysfunction. OCT could be used to investigate experimental treatments that aim to prevent axonal loss in optic neuritis.

Session 18

Infections of the nervous system

O114

Limbic encephalitis: prevalence of paraneoplastic and VGKC antibodies

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Background: Limbic encephalitis (LE) was first described by Brierley and colleagues in 1960. Since then it has been reported as a paraneoplastic syndrome, frequently associated with anti-Hu, anti-Ri, anti-Ma/Ta, anti-CV2/CRMP5, or anti-amphiphysin serum reactivity. We previously reported on a large series of 50 unselected tumor patients with paraneoplastic LE. Symptoms were short-term memory disturbances, epileptic seizures, acute confusional syndrome, further psychiatric symptomes (personality change, hallucinations, depression), brainstem symptoms, signs of hypothalamic involvement, cognition disturbance, and signs of involvement of other neurological systems. Recently, antibodies (ab) against voltage gated potassium channels (VGKC) have been identified as a new marker of autoimmune-mediated LE. VGKC-ab associated LE has been demonstrated to be mostly non-paraneoplastic. However, the impact of these findings remains uncertain, as no data are available so far on the prevalence of VGKC-ab in patients with LE.

Objectives: To determine the prevalence of paraneoplastic ab and VGKC-ab in patients with LE.

Methods: We screened sera of 184 patients with symptoms compatible with the diagnosis of LE sent to our laboratory for paraneoplastic ab testing between 2001 and 2004 for the presence of ab against Hu, Ri, Ma, Ta, and VGKC. Paraneoplastic ab were assessed by indirect immunofluorescence and Western blot. VGKC-ab were detected by radioimmunoprecipitation using 125-I-dendrodotoxin. Cutoff value for VGKC-Ab was 100 pM. In addition, clinical data of ab-positive patients were analyzed.

Results: A classical paraneoplastic marker was found in 29 of our patients (15.7 %), including anti-Hu in 13 (7 %), anti-Ma in 8 (4.3 %), and anti-Ta in 8 (4.3 %) patients. VGKC-ab were detected in 17 patients (9.2 %). VGKC-ab conc > 400 pM as expected in acute LE were present in 8 (4.3 %) patients (median 2623 pM, range 638–5488). In three patients with lowish, but clearly positive VGKC-ab conc (> 100, but < 400 pM), retrospective analysis of previously collected and stored samples was done and revealed high VGKC-ab titers (> 2000 pM) in these patients. A tumour was detected in 25/29(86 %) paraneoplastic and 3/17 (17 %; lung cancer; low titers) VGKC-pos patients.

Conclusions: Our results confirm anti-Hu and anti-Ma/Ta as being the most frequent reactivities in LE. However, we found a striking high prevalence of VGKC-ab in LE patients of ~10 %. VGKC-ab associated LE revealed to be mostly non-paraneoplastic. Titers may decline significantly in the course of the disease.

O115

Tuberculous meningitis – new diagnostic options?

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Objective: To determine new immunological methods to facilitate the diagnosis of tuberculous meningitis.

Background: Tuberculous meningitis is a rare manifestation of tuberculosis with a higher incidence in elderly and immune deficient patients. Due to various differential diagnoses it is often difficult to establish the disease. The standard diagnostic methods, microscopy, bacterial culture and polymerase chain reaction (PCR) for Mycobacterium (M.) tuberculosis not infrequently yield negative results.

Methods: In a patient with chronic meningitis highly suggestive of tuberculous meningitis, the standard diagnostic criteria were not met. In spite of continuous tuberculostatic treatment, clinical symptoms, MRI and CSF findings typical of but not specific for tuberculous meningitis remained virtually unchanged. After 18 months, the patient discontinued medication due to adverse events. Only then, M. tuberculosis PCR became positive.

We applied two immunological methods for diagnostic purposes: (1) CSF and peripheral blood lymphocytes were cultured in the presence of M. tuberculosis proteins (protein purified derivative, PPD) or control antigens. After 5 days, secretion of interferon (IFN)-gamma was measured by ELISA and proliferation was assessed by (methyl-3H)-thymidine incorporation. (2) A phage library expressing randomized heptapeptides was applied to detect the antibody specificity of CSF Ig.

Results: As there was virtually no response to therapy, the diagnosis was questioned for months. The two additional immunological methods supported the presence of tuberculous meningitis from the onset of the disease, more than one year before its proof by M. tuberculosis PCR. (1) Cultured CSF lymphocytes exhibited a high specific proliferative activity and interferon gamma secretion in the presence of PPD. (2) High antibody titers against M. tuberculosis epitopes were observed. M. tuberculosis-specific target antigens could be defined using this method.

Both methods exhibited a high sensitivity and specificity as assessed in other patients with tuberculous meningitis compared to other inflammatory neurological disorders.

Conclusion: Both methods are suggested in patients with an unclear chronic meningitis suggestive of tuberculosis but without proof applying standard diagnostic methods.

O116

The chemokine CXCL13 (BLC): a putative diagnostic marker for neuroborreliosis

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The diagnosis of clinically suspected neuroborreliosis (NB) is based on the presence of lymphocytic pleocytosis and the detection of intrathecally produced Borrelia burgdorferi specific antibodies. However, the antibody production might yet be absent in early stages of the disease and antibiotic therapy has often to be initiated before the results of the antibody index are available. Thus, quickly available CSF markers specific for LNB would be desirable. To identify a characteristic cytokine expression profile in NB which could reveal a diagnostic marker, protein array analysis of pooled cerebrospinal fluid (CSF) samples – to minimize interindividual variability – of acute NB patients was performed and compared with noninflammatory (NIND) and other inflammatory diseases. With this method, a marked (more than twofold) upregulation of eight cytokines in the CSF of NB patients was detected: GRO, IL-6, IL-8, IL-10, IP-10, Light, MIF and BLC. As BLC (CXCL13) has not been reported in inflammatory CNS diseases so far, but was found to be highly upregulated in NB patients, the BLC levels in single CSF samples were reanalyzed by ELISA. ELISA detected BLC in the CSF of every NB patient ($n = 25$) (mean: 3330 pg/ml, equivalent to 218.7 ng/g total CSF protein). In contrast, BLC was below the detection limit in all NIND patients ($n = 34$) (mean: < 3.97 pg/ml or < 1.7 ng/g protein, $p < 0.001$). Treatment of NB-patients with 14d of ceftriaxone iv leads to a reduction of CSF BLC levels ($n = 4$, from 410 to 55 ng/g protein, $p = 0.014$).

Additionally, serum samples of 14 NB and 15 NIND patients were analysed by ELISA. Only a mild difference of BLC-serum values between both groups was found (58.1 in NB vs. 25.3 pg/ml in NIND patients, $p < 0.05$). Combined, these data argue for an intrathecal synthesis of this chemokine.

Finally, the BLC expression was investigated in several other inflammatory CNS diseases (bacterial and viral meningitis, Guillain-Barré syn-

drome, chronic inflammatory demyelinating polyneuropathy, relapsing-remitting and secondary progressive multiple sclerosis, and Bell's palsy) by protein array. BLC was below the detection limit (< 10pg/ml) in all these potential differential diagnoses of LNB.

In conclusion, this study demonstrates that BLC appears to be a putative candidate for an additional diagnostic marker for acute LNB.

O117
Cytotoxic T lymphocytes in viral encephalitis
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It is widely accepted that cytotoxic T cells play a dominant role in viral encephalitis. However, little is known about the specific cytotoxic mechanisms used by these cells. The aim of this study is to determine the role of the various cytotoxic pathways (granzyme B (GrB)/perforin-mediated, Fas/FasL-mediated, Tumor Necrosis Factor (TNF)-mediated) used by CD8+ T cells in acute and chronic viral encephalitis. Here we investigated the (quantitative) distribution of GrB+CD8+ T cells in virus-infected brains, the patterns of activation, cytotoxicity mechanisms and induction of cell death in target cells. We used immunohistochemical and confocal fluorescence double labeling techniques performed on formalin-fixed, paraffin-embedded sections from selected viral encephalitis brains (cytomegalovirus encephalitis (CMV, n = 7), tick-borne encephalitis (TBE, n = 5) and subacute sclerosing panencephalitis (SSPE, n = 2), progressive multifocal leucencephalopathy (PML, n = 8) and HIV- encephalitis (HIVE, n = 4)).

Results show a predominance of CD8+ T cells in the inflammatory infiltrate of all encephalitis cases. We found a high proportion (20–30 %) of GrB cells sustained throughout the course of CMV encephalitis (4–20 weeks). Percentages of GrB positive cells in TBE, PML and HIV-encephalitis were on average fairly constant at about 10 % and in SSPE very low (1 %).

Preliminary studies performed with confocal laser scanning microscope revealed a close contact between CD8+ cells and specified virus targets in all named encephalitis cases. In TBE and SSPE we found close apposition of GrB+ cells to neurons especially in the early course of the disease. These results indicate that especially during the early stage of disease, GrB-mediated cytotoxicity is important in viral encephalitis. This work was supported by a grant from the Austrian FWF (P-16063-B02).

O118
Double infection with encephalitis and Lyme borreliosis transmitted by tick bite
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In endemic regions a single tick bite has the potential to transmit both tick-borne encephalitis (TBE) and Lyme Borreliosis (LB). This retrospective study analyses both the clinical features, and differential diagnosis in 51 patients with evidence of combined tick-borne infection with TBE and LB (30 men and 21 women, av. 50 years old, none vaccinated against TBE). Most (67 %) had professional or leisure exposure to the woodland habitat of ticks, and recorded a preceding tick bite (69 %) or visit to a forest (14 %).

Ninety-eight per cent started with an acute febrile illness compatible with either TBE or LB, biphasic fever suggestive of TBE occurring in 55 %. Meningitis occurred in 92 % of our patients, accompanied by painful radicular symptoms in 39 %. Muscle weakness occurred in 21 (41 %), in 15 (29 %) consisting of the flaccid paralysis compatible with either TBE or LB. Only two presented with the bulbar palsy typical of TBE, one of whom died. Facial palsy typical of LB occurred in only 2. Oculomotor disturbances more typical of TBE occurred in 3. Other features typical of LB were detected in our patients: distal peripheral neuropathy in 4; arthralgia in 9; local erythema 1 to 12 days after tick bite in 7; erythema chronicum migrans in 1; cardiac conduction abnormalities in 15. These patients with double infection with TBE and LB fell in 3 main clinical groups: febrile illness – 3 (6 %); meningitis – 15 (30 %); central or peripheral neurological deficit (meningoencephalitis, meningomyelitis, meningoradiculitis, polyradiculoneuritis) – 33 (65 %). Systemic features pointing to LB.

The clinical occurrence of both LB and TBE varies after exposure via tick bite, and the neurological manifestations of each disorder vary widely, with considerable overlap. This observational study provides no evidence that co-infection produces unusual manifestations due to unpredicted interaction between the two disease processes. Patients with tick exposure presenting with acute neurological symptoms in areas endemic for both LB and TBE should be investigated for both conditions. The threshold for simultaneous treatment of both conditions should be low given the possibility of co-occurrence and the difficulty in ascribing individual neurological manifestations to one condition or the other.

Session 19

Neurogenetics 1

O119
A novel twinkle gene mutation in autosomal dominant progressive external ophthalmoplegia and multisystem failures
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Objective: To describe a family with a novel twinkle gene mutation with adult onset autosomal dominant progressive external ophthalmoplegia (adPEO), multi-organ failure and to study the natural course of illness in this family.

Design: We describe a native Saudi Arabian kindred with affected individual in three generations indicating autosomal dominant inheritance. The proband was the grandmother who presented with progressive PEO which started in her late 30s. In addition, she suffered from pathological obesity, exercise intolerance and recurrent respiratory failure which led to multiple admissions to the Intensive Care Unit with multi-organ dysfunction including hepatopathy, diaphragmatic weakness, with apneic spells, sick sinus syndrome and severe encephalopathy. This clinical syndrome reversed spontaneously after periods of rest, ventilation and nutritional support. She died at age 62 because of encephalopathy and hepatic failure. Two of the daughters had, in addition to PEO and myopathy, respiratory, endocrine and hepatic failure, sick sinus syndrome and recurrent encephalopathy. Examination of 32 subsequent family members and offspring disclosed PEO associated with fatigue in four individuals, respiratory failure in one and hypothyroidism in two.

Results: Muscle biopsies in the four affected persons showed more ragged red fibres in the grandmother (~ 18 %) than in her two daughters (~ 8 % and 11 %) with cytochrome C-oxidase stain negative fibres. Multiple mitochondrial DNA deletions were evident by long-range and real-time polymerase chain reaction, but not by southern blotting. A CTG to GGG change was noted corresponding to a novel heterogenous leu360GIy mutation in a conserved region of the "twinkle" gene. The mutation co-segregate with the clinical phenotype in the family was not found in the 120 control alleles. Screening for this mutation, in the 32 offspring, showed mutation of the leu36GIy in all of the mildly affected individuals and in 10 clinically normal relatives under the age of 30 years.

Conclusion: This family illustrates a new phenotype of adPEO and the presence of reversible multisystem failures associated with a novel mutation in the "twinkle" gene (leu360GIy). This syndrome needs to be added to the growing list of clinical abnormalities associated with PEO.

O120
Autosomal dominant spinocerebellar ataxia type 17: clinical and genetic study of seven Italian families
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SCA17 is a rare type of autosomal dominant spinocerebellar ataxia caused by a CAG/CAA expansion in the gene encoding the TATA-binding protein (TBP). Up to date, repeat expansions in the TBP gene were identified in approximately 40 pedigrees, mostly from Japan and European countries. We screened for CAG expansion in TBP gene 191 subjects with cerebellar ataxia and 87 subjects with suspected Huntington disease, who were negative at the molecular test. Genetic analysis demonstrated the presence of

an expanded allele with ≥45 CAG repeats in ten patients from seven unrelated Italian families. Two patients were sporadic cases, four subjects (2 families) had a positive family history for spinocerebellar ataxia, and four patients (3 families) had a family history for psychiatric and movement disorders. Six patiens carried 45 CAG repeats, one patient carried 46 repeats, while three patients had expansions larger than 50 repeats. In our patients the age at onset ranged from 19 to 55 years, and the age at examination ranged between 25 and 62 years. The first symptoms were characterized by cerebellar gait ataxia and limb incoordination in 4/10 patients (from 2 families), while in the remaining cases the onset was characterized by behavioral disturbances, psychosis and mild choreic movements. Clinical features included cerebellar gait and limb ataxia, dysarthria, cognitive impairment, chorea, dystonia, and psychiatric features. Clinical severity was extremely variable. Five patients showed autonomous walking, three were chair-bound, and two patients were bed-ridden. The latter patients had severe tetraplegia, rigidity of the limbs, anarthria, cognitive decline, and in one case gastral tube feeding was required. Brain MRI, performed in 5 patients, showed marked cerebellar and cerebral atrophy. No deficits of the peripheral nervous system were identified. Electroculographic examination, performed in seven subjects, showed fragmented pursuit in horizontal and vertical planes, and hyperreflexia of VOR. Saccades were slow and hypometric in two subjects and hypermetric in the other cases. None of the patients showed gaze-evoked nystagmus. Severity of the clinical phenotype did not correlate either with the length of the CAG expansion or with disease duration. Our data confirm that CAG expansion in the TBP gene can be associated with variable clinical phenotypes ranging from Huntington disease-like phenotype and autosomal dominant spinocerebellar ataxia.

O121

A novel form of dominant cerebellar ataxia characterised by slow progression and ophthalmoparesis

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We identified a four-generation Italian family (MI-A091) in which several affected members presented cerebellar ataxia transmitted as an autosomal dominant trait. The clinical phenotype was characterized by juvenile onset, slowly progressive cerebellar ataxia, nystagmus and ophthalmoparesis. Twenty-nine family members, including 10/11 living affected subjects, and 19 asymptomatic individuals, were neurologically examined. Considering the patients in all generations, the mean age at onset was 19.5 years (range 12–36). The course of the disease was characterized by very slow progression with most of the patients remaining independently ambulant in their late sixties. The first symptoms were invariably unbalance in standing and mild gait incoordination, followed by slurred speech and oculomotor disturbancies. Increased patellar tendon reflexes were found in 8/10 patients, increased muscle tone in lower limbs in 3/8, and Babinski sign in 4/8. No sensory deficits were recognized at clinical examination. In 5 patients a mild to severe horizontal gaze-evoked nystagmus was present, whereas the remaining patients had slow saccades. Moderate to severe ophthalmoparesis could be recognized in 6 out of 11 patients, and in 5 of them ptosis was associated. Genetic analysis excluded the presence of pathological repeat expansions in SCA1–3, 6–8, 10, 12, 17 and DRPLA genes. RED analysis did not reveal CAG/CTG expansions above 40 repeats. Two-point and multipoint linkage LOD scores excluded the linkage at SCA4, SCA5/20, SCA11, SCA13–16, SCA18, SCA19/22, SCA21, SCA25, and FGF14-SCA loci. On the basis of the clinical phenotype and of the results of genetic analyses, we propose that this disorder represents a novel form of autosomal dominant spinocerebellar ataxia.

O122

Gln192Arg polymorphism of paraoxonase-1 gene and the risk for amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder. Epidemiological studies suggest that exposure to agricultural chemicals (pesticides/herbicides) as far as 20 years before the onset of disease may be a risk factor for the development of ALS.

Paraoxonase-1 is a serum enzyme that plays a role in organophospate metabolism and LDL oxidation. Its concentration and activity is modulated by a common Gln192Arg polymorphism in the paraoxonase-1 (PON1) gene localized on chromosome 7. Homozygotes for Arg allele display rapid hydrolysis of paraoxon and slow of diazoxon, environmental chemicals used in agriculture.

The present study was designed to analyse a possible association between PON1 Gln192Arg polymorphism and the risk of ALS in a case-control study.

We included 128 patients with sporadic ALS and 238 healthy controls matched for age and sex. The definite or probable diagnosis of ALS was established according to El Escorial Criteria (1994). PON1 Gln192Arg polymorphism was detected by PCR and restriction enzyme digestion.

The study has shown that both the distribution of the genotypes (cases: Gln/Gln – 58, 45 %; Gln/Arg – 52, 41.1 %; Arg/Arg – 18, 14 %, controls: Gln/Gln – 130, 55 %; Gln/Arg – 91, 38 %; Arg/Arg – 17, 7 %) ($p = 0.032$) and alleles (cases: Gln – 168, 66 %, Arg – 88, 34 %, controls: Gln – 351, 74 %, Arg-125, 26 %) differ significantly between cases and controls ($p = 0.03$, and $p = 0.02$ respectively).

We have found that Arg/Arg genotype and Arg allele of PON1 Gln192Arg polymorphism is a genetic risk factor for ALS, which may suggest the relationship between diazoxon exposure and the risk for ALS.

O123

Generalised dystonia with severe cerebellar atrophy is a new inherited dystonia phenotype: a study of eight families

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Inherited dystonias constitute a heterogeneous group of diseases with a wide range of phenotypes caused by many different genes or loci (DYT1–14). None of these forms are associated with cerebellar ataxia or atrophy.

We describe twelve patients from eight families with a new "dystonia-plus" phenotype associated with cerebellar atrophy. All had severe dystonia associated with mild cerebellar signs but marked cerebellar atrophy on brain MRI. Mean age at onset was 28.1 ± 11.3 years (range: 9–42 years), and the mean disease duration at examination was 15.7 ± 8.6 years (range: 2–30). At onset, dystonia was focal, mainly affecting the hand, neck and facial muscles. Later on, dystonia spread to lower limbs and/or to pharyngolaryngeal muscles, leading to severe dysphagia and dysphonia. Cerebellar ataxia was limited to unsteadiness and progressed very slowly at most. The paucity of clinical cerebellar signs contrasted with the marked cerebellar atrophy on brain MRI. The observation of two affected sibs in four families, and one family with probable consanguinity, support the hypothesis of a genetic basis. The predominance of affected males is compatible with X-linked inheritance. Biological, metabolic investigations and brain MRI excluded the major causes of dystonia and cerebellar ataxia. The major genes responsible for hereditary dystonias, autosomal dominant and recessive cerebellar ataxias were excluded by molecular analyses.

We postulate that this disorder probably represent a new genetic entity among the hereditary dystonia. We propose to designate this phenotype DYTCA for Dystonia with Cerebellar Atrophy, the two consistent findings in our patients.

O124

Mitochondrial DNA deletions in dementia with Lewy bodies – A single neuron study

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Introduction: Dementia with Lewy bodies (DLB) is the second most frequent cause of degenerative dementia in the elderly and has strong clinical and neuropathological similarities with Parkinson's disease (PD). Increasing evidence links PD with mitochondrial dysfunction. Mitochondrial DNA (mtDNA) deletions, such as the common deletion (CD), accumulate with aging in the brain, but so far their role in the pathogenesis of PD remained uncertain. We employed single cell techniques to test the hypothesis that DLB is associated with mitochondrial dysfunction due to accumulation of mtDNA deletions. Evaluating brains from DLB rather than PD patients has the advantage that neuronal loss is less pronounced in the former, leaving enough neurones to be sampled.

Materials and Methods: SN from 12 DLB cases and 7 matched controls was stained for cytochrome C oxidase (COX) and succinate dehydrogenase. COX normal (COXnorm; suggestive of normal mitochondrial function) and COX deficient (COXdef; suggestive of mitochondrial dys-

function) single SN neurones (n = 658) were captured by Laser-Microdissection and their deletion levels were measured by a real-time PCR method, which allows the quantification of the majority of mtDNA deletions in single cells.

Results: The density of COXdef neurones was increased 3.8-fold in DLB patients versus controls. High levels of deletions were found in DLB patients and in controls with a trend towards higher levels in patients (52.3 ± 9.3 % vs. 43.3 ± 9.3 % in controls; p = 0.06). COXdef neurones contained higher levels of deletions than COXnorm cells (66.9 ± 19 % vs. 47.7 ± 24 %, respectively; p < 0.00001).

Discussion: Our data provide direct evidence that in single neurones, mtDNA deletions can accumulate to levels which are sufficient to produce mitochondrial dysfunction in aging and in DLB. Previous studies on brain homogenates from PD patients were not conclusive in this point and reported much lower deletion levels (max. 12 %). This highlights the necessity for molecular biological studies to be carried out on the highly specific single neurone level and for quantification methods which are not limited to one specific type of deletion. In conclusion, our results provide further evidence for an involvement of mitochondrial dysfunction in the pathogenesis of DLB. Our observations pose some interesting questions as regards the mechanism of the accumulation of deleted mtDNA in neurones and the role of the deleted mtDNA on neuronal cell death.

Session 20

Neurogenetics 2

O125

Identification and characterisation of hCOX18 and hCOX19, two new human genes involved in cytochrome C oxydase assembly

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Background: The biogenesis of Cytochrome c oxidase (COX), the complex IV of the mitochondrial respiratory chain, is a very complicated process controlled by two distinct genomes: mitochondrial and nuclear DNA. It requires structural proteins and several accessory proteins involved in prosthetic group biosynthesis, transport and insertion of metal cofactors and the correct assembly of different intermediates to form the holoenzyme. Mutations in these so called "COX assembly genes" are the main cause of isolated COX deficiency in humans. 30 genes are known to participate to this process in yeast while only 10 human homologues have been identified so far. Among the yeast (y) genes, yCOX 18 and yCOX19 seem required for the expression of COX: the first one being involved in the export of the mitochondrial encoded Cox 2p C-tail, while the latter has a role in copper metabolism and COX assembly.

Methods: using the corresponding yeast sequences as probe we identified in Gene bank database the human homologues of COX18 and COX19 (hCOX18, hCOX19) and we cloned them. The cDNA was used a) to perform a Northern blot to study the tissue distribution of the proteins and b) to generate a fusion protein in order to study their cellular localisation.

The study of the hydrophobic profile and the predicted subcellular localisation was performed with specific software (SOUSI, PREDOTAR, MITOPROT).

Results: hCOX18 gene is localized on chromosome 4q21.1 and code for a 333 amino acids mitochondrial protein, while hCOX19 gene is on chromosome 7p22.3 and code for a 90 amino acid protein preferentially present on cytosol. Both of these genes are expressed ubiquitously, but hCOX 18 present a higher expression in liver. At least two different transcripts were detected for hCOX18 (2 and 3.4kb), while several transcripts are present of hCOX 19 the smallest being a 3.5kb transcript.

Conclusion: the identification and characterisation of these two new human genes that could be involved in COX assembly process will allow us to study a series of patients presenting with isolated COX deficiency without any mutations in know COX assembly and structural genes. These findings will maybe open a new perspective in the diagnosis of this rare but dramatic disorder.

O126

PCWH–a novel complex neurocristopathy caused by SOX10 mutations

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We identified a novel complex neurocristopathy, PCWH, which is characterized by a combination of four independent syndromes, Peripheral demyelinating neuropathy, Central dysmyelinating leukodystrophy, Wardenburg syndrome, Hirschsprung disease and demonstrated that mutations in SOX10 gene are responsible for PCWH. SOX10 mutations also cause a non-neurological syndrome, Wardenburg-Hirschsprung disease (WS4). The purpose of this study is (i) to evaluate the variation of clinical features and severity, and (ii) to determine the molecular basis for genotype-phenotype correlations.

First, we summarized clinical features of 12 cases of PCWH including our own 7 cases. SOX10 mutations were identified in all cases. Peripheral neuropathy varies from lethal severe congenital hypomyelinating neuropathy to mild demyelinating neuropathy. Leukodystrophy ranges from severe dysmyelinating leukodystrophy with complete absence of myelin to very mild leukodystrophy. Wardenburg syndrome and Hirschsprung disease are less variable among the patients.

Next, we determined the genotype-phenotype correlations. Most SOX10 mutations resulted in premature termination codons (PTCs). PCWH-causing mutations are exclusively accumulated in exon 5, the final exon. In contrast, those causing WS4 are found in exon 3 and 4. A polarity of PCWH-causing mutations in exon 5 was also noted. Thus, an apparent cause-and-effect relationship between the mutations and the disease state was identified. In vitro functional assays suggested that (i) WS4 is caused by PTCs that activate nonsense-mediated mRNA decay (NMD) pathway, thus haploinsufficiency is the likely mechanism, and (ii) PCWH is caused by either PTCs that escape NMD pathway allowing the production of dominant-negative truncated proteins, or extension of SOX10 proteins that potentially act as gain-of-function alleles.

O127

New mutations in the HSN2 gene in two cases of HSAN type II

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Hereditary Sensory and Autonomic Neuropathies (HSAN) are rare disorders of sensory (and autonomic) neurons. HSAN type II is characterized by autosomal recessive inheritance, early onset, severe sensory loss to all modalities, painless ulcers, neuropathic arthropathy and acromutilations. Recently, mutations in the HSN2 gene on chromosome 12p13.33 have been reported in 6 families of different ethnic origin. All are frameshift or nonsense mutations resulting in premature protein truncation. We report 2 males aged 32 and 51 years with HSAN II from Italy and Austria, respectively. There was no known consanguinity of the parents. Difficulties in manipulation were noted at age 6 months in the Italian patient, when deep tendon reflexes were reduced. At age 3 distal sensory loss was evident. He then developed recurrent hand and foot ulcers. Painless pathologic fractures occurred, involving first the toes, then metatarsal and tarsal bones, and at age 15 the right knee. At repeated examinations, he had anaesthesia to all modalities distally and hypoesthesia proximally in all limbs; he also had trunk hypoesthesia; deep tendon reflexes were absent. He suffered from progressive amputations extending from fingers and toes to both hands, left leg, and right thigh. Nerve conduction studies were normal in motor nerves, but no sensory response could be obtained in upper and lower limbs. A similar phenotype but with later disease onset was observed in the Austrian patient. Nerve biopsy in the Italian patient at age 8 revealed complete loss of myelinated fibers, presence of unmyelinated fibers, and endoneural fibrosis. We performed direct sequencing of the coding region of HSN2 in the patients and available family members. The Italian patient resulted to be a compound heterozygote for two frameshift mutations. The first mutation is a c.254delC causing a frameshift in codon 85 leading to a premature stop codon at position 98 (Pro85fsStop98). This mutation is also found in the mother and the unaffected brother. The second mutation is a c.1089_1090insT causing a frameshift in codon 364, resulting in another premature stop codon at position 379 (Gln364fsStop379). This mutation is also present in the father. The Austrian patient was homozygous for a nonsense mutation c.550C > T introducing a stop codon at position 184 (Gln184Stop). The three HSN2 mutations are different from those previously reported.

The first 3 authors contributed equally to this work.

O128

A novel presenilin 1 mutation associated with early onset Alzheimer's disease and spastic paraparesis

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Mutations in the presenilin 1 (PSEN1) gene on chromosome 14 are the most frequent cause of early onset familial Alzheimer's disease (EOFAD). In most of the families the affected individuals present with classical AD without spastic paraparesis. Progressive dementia associated with spastic paraplegia caused by PSEN1 mutation has been described in several families. We report the clinical features and molecular genetic findings of a woman who developed early onset of dementia with spastic paraparesis, which was associated with a novel PSEN1 mutation in exon 8 (Arg278Ser). The family history was compatible with autosomal dominant inheritance.

The proband is a 44-year-old right-handed woman, presenting with a 5-year history of progressive difficulties with walking and stiffness in her legs, poor balance and slurred speech. She developed memory impairment within 12 months of her gait disorder. Neurological examination revealed lower limb spasticity and weakness with bilateral Babinski signs. Her cognitive evaluation revealed impairment of visual and verbal anterograde memory. By the age of 44 years she was wheel-chair dependent and her Mini-Mental State Examination was 18/30. Magnetic resonance imaging of the brain and spinal cord showed mild-moderate cerebral and cerebellar atrophy without parenchymal signal change and normal cord. Four of the proband's close relatives in two generations had similar neurological problems associated with reduced life expectancy (39–42 years). Her PSEN1 mutation analysis of exons 4–12 and adjacent splice sites showed a novel nucleotide transversion in exon 8 and this was confirmed by fluorescent DNA sequencing. This mutation is predicted to substitute a serine for an arginine residue at position 278 (Arg278Ser) in the PSEN1 polypeptide.

The novel PSEN1 mutation identified in this patient adds to the diverse list of existing mutations causing EOFAD associated with spastic paraparesis. PSEN1 mutations are thought to cause altered processing of amyloid precursor protein resulting in increased formation of the more amyloidogenic species A beta 42 compared to the more commonly occurring A beta 40. It is thought that very high levels of A beta 42 production are implicated in the phenotype of spastic paraparesis as well as dementia.

O129

Clinical and genetic study of a large SPG4 Italian family

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Background: As well as spastic paraplegia, other clinical features such as peripheral neuropathy, cognitive impairment, and urinary disturbances have been reported in SPG4. However, it remains unknown whether there is a correlation between disability and time since onset of symptoms.

Objective: To perform clinical investigations to evaluate the extension of the neurodegeneration in one SPG4 family, in which all the affected members share the same frame-shift mutation in the spastin gene.

Methods: Neurological evaluations, neurophysiological and neuropsychological examinations, neuroimage studies, serum total testosterone (TT) level assessment, urological investigations, linkage study, and sequence analysis of the SPG4 gene.

Results and Discussion: A novel SPG4 906delT frame-shift mutation in exon 6 was identified in a large Italian family with an autosomal dominant form of hereditary spastic paraplegia (ADHSP). Intra-familial phenotypic variations observed in the pedigree included: spasticity and additional clinical features, such as peripheral sensory-motor neuropathy, cognitive impairment, and urological dysfunction. Severe clinical features were found predominantly in the men who were affected and there was no statistically significant correlation of disability and time since onset of symptoms, suggesting the existence of other genetic/non genetic modifier(s) including gender.

Session 21

Multiple sclerosis 3

O130

Longitudinal fMRI changes following tumour-like lesions in multiple sclerosis

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Background: Movement-related cortical adaptive functional changes have been shown in patients with stable MS independent of the disease phenotype. Fewer studies have assessed the short-term fMRI changes after an acute relapse.

Objective: To assess the movement-associated cortical changes following an acute motor relapse secondary to a single tumor-like lesion in MS patients, in order to investigate the role of cortical reorganization during clinical recovery and to evaluate the relationship between the degree of recovery and longitudinal fMRI changes in motor areas.

Patients and methods. We recruited 12 right-handed patients after a clinical attack involving the motor system secondary to a large demyelinating lesion. We obtained fMRI during repetitive flexion-extension of the last four fingers of the impaired and unimpaired hand. In four patients a longitudinal fMRI study was also performed (mean follow-up duration = six months). FMRI data were analyzed using SPM99. Hand motor performance was assessed using the nine-hole peg test (NHPT).

Results: The primary sensorimotor cortex (SMC) of the ipsilateral hemisphere was significantly more activated during task performance with the impaired hand than with the unimpaired hand. On the contrary, during task performance with the unimpaired hand, the ipsilateral cerebellum and several motor areas in the contralateral hemisphere, including the primary SMC, the secondary sensorimotor area and the thalamus were significantly more active. Tumor-like lesion volume was significantly correlated with relative activation of the primary SMC, bilaterally ($r = -0.86$ and -0.85), whereas NPTH score was significantly correlated with relative activation in the primary SMC of the affected hemisphere ($r = 0.88$).

Longitudinal fMRI examinations showed a progressive recovery of function of the primary SMC of the affected hemisphere in the two patients with a clinical improvement. On the contrary, in the two patients who did not show any clinical recovery, there was a progressive recruitment of several regions in the frontal lobes, bilaterally.

Conclusion: Tumor-like MS lesion affecting the motor system can determine short- and medium-term cortical changes mainly characterized by the unmasking of pathways in the unaffected hemisphere. The regain of function of "classical" motor areas of the affected hemisphere seems to be a critical step for the subsequent clinical recovery.

O131

Dysferlin as a marker for blood brain barrier damage

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Dysferlin is a 237 kD protein of the muscle cell membrane. It contains six C2-domains which are known to play a role in vertebrate inflammatory pathways. In humans, a mutation within the dysferlin gene leads to two clinically distinct muscular phenotypes: Miyoshi myopathy and Limb girdle muscular dystrophy.

In this study we describe that dysferlin is expressed on fenestrated endothelial cells of blood vessels and on endothelia of lymphatic capillaries. Brain endothelial cells are dysferlin negative except those in the choroid plexus and the circumventricular organ. In animals with autoimmune encephalomyelitis (EAE), massive dysferlin expression was found in endothelial cells in active lesions, which correlated with the severity of the clinical disease. Based on these observations we studied dysferlin expression in 80 MS lesions from different clinical subtypes as well as in 12 normal controls and 7 controls who died of systemic infectious diseases.

Only few vessels of both control groups were dysferlin positive. In contrast, in actively demyelinating MS lesions numerous endothelial cells showed reactivity for dysferlin. In inactive lesions, dysferlin expression was significantly lower compared to active lesions, but still higher than in controls. Expression of dysferlin in endothelial cells correlated with the degree of blood brain barrier damage reflected by fibrin extravasation. According to our data, dysferlin may be the first marker which allows to directly visualize blood brain barrier dysfunction at the level of endothelial cells.

O132

The pathology of progressive multiple sclerosis

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The clinical course of multiple sclerosis is characterized by relapses and progressive deterioration of neurological function. While the symptoms of patients with relapsing-remitting MS can be explained by focal white matter lesions in the CNS, this is not the case for patients with primary or secondary progressive MS who experience a gradual accumulation of their clinical deficit. So far no pathological feature of the disease has been described which clearly distinguishes relapsing-remitting from progressive disease in MS patients.

In the present study, we systematically analyzed cortical and white matter pathology in a large sample of multiple sclerosis brains with different disease courses (11 cases with acute MS, 6 with RRMS, 20 with SPMS and 14 with PPMS). Hemispheric and double hemispheric tissue sections were examined for cortical demyelination and pathological changes in the white matter which offered the unique opportunity to evaluate disease involvement of large tissue areas.

Cortical demyelinated plaques were abundant in patients with primary or secondary progressive MS, but were rare in patients with acute or RRMS (percentage of demyelinated cortical area: acute MS: 0.36; RRMS: 4.54; PPMS: 14.89; SPMS: 21.22; $p = 0.0014$). Focal load of demyelinated lesions in the white matter was almost the same in the four groups.

Similar results were obtained from our analysis of the cerebellar cortex (percentage of demyelinated cortical area: acute MS: 0.85; RRMS: 1.89; PPMS: 33.28; SPMS: 38.2; $p = 0.042$).

Especially in primary, but also in secondary progressive MS cases, myelin pallor was observed in the normal appearing white matter, which was associated with significant inflammation as well as microglia and macrophage activation. These pathological changes were sparse in acute and RRMS brains (inflammatory infiltrates per mm^2: acMS: 0.05; RRMS: 0.07; PPMS: 0.20; SPMS: 0.30; $p = 0.019$). No correlation between size and location of white matter plaques with cortical demyelination or diffuse white matter damage was observed.

In conclusion, we suggest that in MS brains three different pathological processes occur, which occur at least in part independently from each other: focal white matter plaques, cortical demyelination and diffuse damage of the white matter.

O133

Raised white matter myo-inositol in patients with clinically isolated syndromes is associated with the development of multiple sclerosis

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On proton MR spectroscopy (MRS), myo-inositol (Ins) is elevated in the normal appearing white matter (NAWM) of patients presenting with clinically isolated syndromes (CIS) suggestive of multiple sclerosis (MS)1. The aim of this study was to see whether the Ins increase is related to the subsequent development of clinically definite MS (CDMS).

Methods: Sixty seven CIS patients (57 optic neuritis, 5 brainstem, 5 spinal cord syndromes, 44 female, 23 male, median age 33 years) underwent MRS a mean of 19 weeks (standard deviation (SD) 3.4) after the onset of clinical symptoms. Patients were followed up for 3 years or until they developed CDMS before the 3 year time point. Forty six healthy controls (24 female, 22 male, median age 36 years) also had MRS. In all cases, single voxel MRS was acquired from NAWM in the posterior parietal centrum semi-ovale region. The MRS acquisition was a PRESS sequence with a TR 3000 ms, TE 30 ms and 192 averages. Concentrations of total N-acetyl aspartate (tNAA), total creatine and phosphocreatine, choline, glutamate and glutamine and Ins were estimated using the LC Model. Differences between patients and controls were assessed by multiple regression of metabolite concentrations on a binary disease status variable and age and gender covariates to control for any potential confounding effects.

Results: Thirty eight (57 %) of the patients developed CDMS by the Poser criteria and 44 (66 %) developed MS by the McDonald criteria. Ins was significantly higher in the NAWM of the CIS patients who developed CDMS (mean 4.00 mM, SD 1.04) when compared with controls (mean 3.31 mM, SD 0.84, $p < 0.00001$) and with patients who did not have CDMS after 3 years (mean 3.29 mM, SD 0.77, $p < 0.001$). Patients with a high concentration of NAWM Ins were more likely to convert to CDMS and at a faster rate than those with lower levels of NAWM Ins. Ins was also significantly higher in those who developed MS by the McDonald criteria (mean 3.96 mM, SD 1.08) when compared with controls ($p = 0.001$) and with patients who did not have McDonald MS at 3 years (mean 3.2 mM, SD 0.78 $p = 0.007$). The other metabolites were not significantly different in CIS NAWM.

Conclusions: In patients presenting with CIS, NAWM Ins is higher in those who develop CDMS within 3 years. Since Ins is a marker of glial cells this study suggests that glial proliferation in the NAWM may be related to the early clinical course of MS.

Reference

1. Fernando et al. (2004) Brain 127:1361–1369

O134

High-dose glucocorticoid treatment for multiple sclerosis relapses enhances the proportion of circulating CD4+CD25+ T cells

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Objective: To determine whether there is a decrease in the number of T regulatory cells at the time of an MS relapse and whether glucocorticoids increase the numbers of these cells.

Background: The subset of CD4+CD25+ regulatory T cells have been shown to be essential for the control of autoimmunity. Deficits in these cells can lead to autoimmunity. Glucocorticoids enhance the number and function of Treg cells in vitro but their effects in vivo are not known. In multiple sclerosis (MS), CD4+CD25+ regulatory T cells (Treg cells) have been shown to have functional but not numerical deficits. However, whether steroid treatment during relapses can increase the pool of Treg cells is not known.

Materials and Methods: Serial measurements of the percentage of CD4+CD25+ T cells were obtained in 5 patients with relapsing-remitting MS during relapse, 48h after i. v. methylprednisolone (1 g/day) and 6 weeks following the course of steroids. The 5 patients in relapse were also compared with a group of 28 stable MS patients.

Results: There was no difference in the percentage of CD4+CD25+ T cells between MS patients and controls or between patients with stable MS and those in remission. There was a significant ($p = 0.022$) increase in the percentage of CD4+CD25+ T cells following 2 courses of 1 g each of i. v. methylprednisolone treatment. There was a return to baseline ($p = 0.043$ compared with during steroids) at 6 weeks, which corresponded to clinical stabilization.

Conclusion: Glucocorticoid treatment for MS relapses transiently enhances the percentage of CD4+CD25+ T cells. Further studies are needed to determine whether this increase contributes to the process of clinical recovery.

O135

Anticipation of age at onset in multiple sclerosis

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Introduction: Approximately 70 % of multiple sclerosis (MS) cases arises between the ages of 20 and 40 years. It has recently been reported that age at onset of MS in Sardinian patients decreases progressively from older to younger generations.

Goals: To assess age at onset in MS during the last 60 years in the region of Costa of Ponent (Barcelona) and to determine whether there is any anticipation of age at onset in our MS patients.

Methods: We use the European database for MS to register and prospectively follow up our patients. A total of 1100 patients were included in the study. All of them met definite or probable MS criteria according to Poser. They were divided into six groups according to decades of birth: subjects born before 1940, from 1940 to 1949, from 1950 to 1959, from 1960 to 1969, from 1970 to 1979 and those born from 1980 on. We performed a survival analysis of age at onset.

Results: Mean age at onset of the whole cohort progressively decreased from the most remote to the most recent decade of birth, being 46 years in those born before 1940 and 42, 35, 28, 24 and 19 years in the following decades ($p < 0.0001$).

In the RR group, mean age at onset was 43 years in the most remote decade and 41, 34, 28, 24 and 19 years in the following decades ($p < 0.0001$).

In the PP group, mean age at onset was 53 years in the most remote decade and 46, 40 and 30 years in the following decades ($p < 0.0001$).

Analysis of the sub-cohort of patients with definite MS showed the same temporal trend ($p < 0.0001$).

Discussion: We found progressive anticipation of age at onset of MS in our patients. There is only a previous report assessing the same phenomenon in Sardinian MS patients. The authors hypothesized that a permissive genetic structure along with changes in the environment might be responsible for the anticipation. Because our population does not have the highly homogeneous genetic make-up of the Sardinian population, it is more likely that environmental changes might contribute to the progressive lowering of age at onset of MS.

Session 22

Multiple sclerosis 4

O136

Health-related quality of life in patients with early multiple sclerosis in the BEST study

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Patients' experience with multiple sclerosis (MS) and its therapy do not always clearly correlate with clinical outcomes. Standardized and validated Patient Reported Outcomes (PRO) such as the Functional Assessment of MS (FAMS) questionnaire help to understand how individual patients value relevant aspects of their condition. Subscales of the FAMS are mobility, symptoms, emotional well-being, thinking/fatigue, general contentment, family/social well-being, and additional concerns. The FAMS comprises 58 items and is available in 28 languages. A higher total score represents better health-related quality of life (HRQoL). In addition, the instrument allows assessment of overall physical health as reported by patients, using the FAMS Trial Outcome Index (FAMS-TOI). The FAMS-TOI comprises the dimensions mobility, symptoms, thinking/fatigue, and additional concerns.

BEST (Betaferon(R) in Early RRMS Surveillance Trial), a prospective international 5-year observational study to assess safety, tolerability and long-term effect of IFNB-1b treatment on early MS disease progression, is now ongoing. More than 3,000 patients from 32 countries speaking 21 languages are included worldwide. Clinical variables, adverse events, and PRO are assessed at baseline and every 6 months. Progression is defined as change in the EDSS by ≥1 step. HRQoL is one of the key outcomes in this observational study.

Interim one-year HRQoL data are available for 343 patients from 8 countries. PRO information was obtained at baseline and at least once post-baseline. HRQoL of the entire sample remained stable over time. Overall physical health as reported by patients was analysed using the FAMS-TOI. The median score from baseline to the individual's last visit was stable (114.3 at baseline versus 116, maximum 148). In the 90 % of patients without progression (287/319), the mean (median) change in FAMS-TOI was +2.8 (+1.4), whereas in the 10 % (32/319) who progressed the score was reduced by 9.4 points. Ongoing analysis includes 2-year follow-up data of these patients.

FAMS was used for the first time in a large international group of patients treated under real life conditions. Easily used in a routine manner, the FAMS seems to discriminate between different courses of MS as well as different levels of disability, including overall physical health as reported by patients.

O137

Sustained effect of glatiramer acetate on cerebral axonal recovery in relapsing-remitting MS: results after three years of serial brain magnetic resonance spectroscopy examination

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Objective: To determine the effect of glatiramer acetate (GA) on cerebral axonal recovery in relapsing-remitting MS (RRMS) patients.

Background: It is postulated that GA induces bystander suppression of inflammation inside the CNS by way of centrally-mediated Th2 responses. We have previously reported results of an ongoing serial brain magnetic resonance spectroscopy (MRS) study after two years of therapy in a small cohort of RRMS treated with GA and compared with untreated RRMS patients. We now report results after three years of follow up.

Design/Methods: We performed combined MRI/MRS on 18 treatment naive RRMS patients before starting therapy with GA and annually thereafter. Four RRMS patients on no treatment were also studied annual MRI/MRS scans. EDSS scores were obtained every six months on all patients and for confirming a relapse. MRS intensities of NAA relative to creatine (Cr) were measured in a multi-voxel volume-of-interest (VOI) centered on the corpus callosum that predominantly contained white matter. We examined the entire VOI as well as white matter appearing normal (NAWM) on conventional MRI.

Results: Baseline (n = 18) mean age, disease duration, and EDSS were 43.5 years, 8.2 years, and 2.77, respectively. Baseline (n = 18) mean NAA/Cr in the entire VOI was 1.97 (± 0.24) and 2.075 (± 0.30) in the NAWM. After 3 years of follow up, 15 of the 18 patients in the treated group were still participating in the study. NAA/Cr ratio remained stable during the third year and significantly improved compared to baseline and the untreated group. Of the 4 untreated patients, 2 patients began therapy with GA during the third year after remaining untreated for the first two years. The patients who began treatment during the third year of follow up showed improvement in their NAA/Cr ratio compared to their own NAA/Cr ratio during the first two years of no treatment. The two patients who remained untreated during the third year continued to show a decline in their NAA/Cr ratio. Detailed analysis will be presented.

Conclusions: Our data suggest a sustained beneficial effect of GA on cerebral axonal injury in RRMS. These findings support a centrally mediated effect of GA and potential neuroprotection.

O138

Risk of myelodysplastic syndrome in long-term treatment with azathioprine in multiple sclerosis

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Azathioprine (AZA) is a widely used immunosuppressive treatment for Multiple Sclerosis (MS). The efficacy compared to immunemodulatory therapies like interferon beta (IFNB) preparations is unclear. Recently, concepts for combination therapy of IFNB and AZA have evolved. An increased risk of mainly solid cancers has been suggested under higher cumulative dosages of AZA. Myelodysplastic Syndrome (MDS) is a rare clonal stem cell disease (incidence 1/100,000 per 10 yr, age group of 40–50) characterised by changes of hematopoesis and cytopenia.

Course is usually benign. Secondary MDS (sMDS) e. g. due to chemotherapy are known. Recently, a 100 fold increased risk of developing AZA-related sMDS in patients treated for non-malignant disorders was reported. Median survival was 9 months only.

Methods: Questionnaires were sent to 636 patients who had been treated in our clinic with the diagnosis of MS to identify those exposed to AZA, known MDS and other malignancies. Complete blood counts were obtained for all patients and analyzed.

Results: 47 % of questionnaires were returned (n = 299, mean age = 44.8 years). 69 patients have been treated with AZA (mean age = 45.6, mean cumulative dosage = 268 g, range 19.5–1050 g). We identified one female (45 yrs, cumulative AZA dose 627 g) with sMDS who was recently treated with BMT. Blood examinations of the other patients were without suspicious abnormalities. 5 additional malignancies were documented in the AZA group (n = 69, 1 oropharyngeal, 1 scrotal, 1 mammary and 2 uterus malignancies) vs. 5 in the non-AZA group (n = 230, 3 mammary carcinomas, non-hodgkin lymphoma, acute myeloic leucemia). Both groups were comparable as to age (48.8 vs. 42.4 years). Mean cumulative dose in patients with malignancies was higher than in the patients without malignancies (324 g).

Conclusions: We identified one case of sMDS related to AZA therapy in a rather small cohort of MS patients exposed to AZA. As the incidence of MDS is usually very low in the examined age group, this is a striking finding.

We confirm recent reports of a considerable risk of AZA-related sMDS. It is strongly recommended to fully evaluate persistent cases of cytopenia with regard to MDS, especially as the course of AZA-related sMDS is severe.

Secondly, the prevalence of malignancies of patients with AZA therapy was associated with higher cumulative dosages and significantly higher than in the non-AZA group which is consistent with previous reports. We doubt that AZA can still be considered a first line therapy in MS.

O139

Long-term follow-up of patients treated with glatiramer acetate: a multicentre, multi-national extension of the European/Canadian double-blind, placebo-controlled, MRI-monitored trial

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Objective: To investigate the long-term evolution of clinical and MRI findings in patients with relapsing-remitting (RR) multiple sclerosis (MS) who participated in the glatiramer acetate (GA) 9003 trial, in order to explore the correlates of long-term GA treatment.

Background: GA is effective in reducing clinical and MRI activity in RRMS. Serial MRI data on a long-term basis were never obtained for large samples of GA-treated patients.

Design/Methods: The 9003 study consisted of two consecutive phases, each lasting nine months. The first treatment phase was randomized, double-blind and placebo-controlled. The second was an active treatment phase for all patients. Treatment consisted of daily administration of 20 mg GA subcutaneously. All patients underwent brain MRI at screening (to be included, they had to have one or more enhancing lesions), baseline, every month during the double-blind phase and every three months during the open-label phase. Clinical assessment included neurological visits with Expanded Disability Status Scale (EDSS) score rating. For the long-term follow-up (LTFU), dual echo, pre- and post-gadolinium T1-weighted brain MRI scans were obtained with the same acquisition scheme as for the original trial and a neurological assessment was performed. Total T2-hyperintense and T1-hypointense lesion volumes, as well as normalized brain volumes (NBV) and percentage BV changes (PBVC), were measured.

Results: One hundred and forty-two of 224 patients who completed the 9003 study (63.4 %) underwent the LTFU after a mean period of 5.8 years. Seventy-three of them had been treated with GA since the study initiation. At 9003 baseline, there were no significant differences between patients who subsequently came at LTFU and those who did not. Among the former ones, MRI measures of disease burden and activity, as well as brain volume changes, at LTFU did not significantly differ between patients originally assigned to placebo and those who were always treated with GA. The proportion of patients who did not reach relevant locomotor disability (EDSS ≥6.0) at LTFU was significantly greater in patients treated with GA during the first nine months of the 9003 trial ($p = 0.03$). PBVC between baseline and LTFU was significantly correlated with T2 lesion volume at study entry.

Conclusions: This study indicates that the earlier initiation of GA treatment in patients with active RRMS might have a favorable impact on the long-term disease evolution.

O140

A phase II randomised, double-blind, placebo-controlled study to evaluate the preliminary efficacy and safety of abatacept, a selective co-stimulation modulator, in patients with relapsing-remitting multiple sclerosis

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Background: Abatacept selectively modulates the co-stimulatory signal required for the full activation of T-cells expressing CD28. Abatacept has demonstrated efficacy in experimental autoimmune encephalomyelitis, a surrogate animal model thought to be predictive of human multiple sclerosis (MS).

Objective: To evaluate the efficacy and safety of abatacept in patients with relapsing-remitting MS (RRMS).

Methods: This was a randomized, double-blind, placebo-controlled cohort study in RRMS male and female patients aged 18–58 years. Patients had definite MS, an Expanded Disability Status Scale (EDSS) ≤6 (inclusive), at least 1 relapse in the preceding 2 years (clinically stable for 2 months prior to treatment) and at least 1 gadolinium T1 (GdT1) enhancing lesion on magnetic resonance imaging. Patients were randomized into 1 of 3 cohorts receiving: abatacept 2 mg/kg, abatacept 10 mg/kg or placebo by infusion on Days 1, 15, 29, and then every 4 weeks until Day 197.

Results: The study terminated early due to an increase in patients with ≥5 new GdT1 lesions in the abatacept 2 mg/kg group. The cohort design resulted in excess patients in the abatacept 2 mg/kg group with poor baseline prognostic factors; 80 % of patients with > 10 GdT1 enhancing lesions were randomized to abatacept 2 mg/kg. A total of 127 of a planned 219 patients were randomized and received ≥1 infusion of study medication. Compared with abatacept 2 mg/kg and placebo, abatacept 10 mg/kg-treated patients had fewer new (1.5 vs. 8.0 vs. 5.5) and total GdT1 enhancing lesions (3.0 vs. 13.0 vs. 8.5), fewer protocol defined exacerbations (20.6 vs. 56.6 vs. 30 %), a lower mean annualized relapse rate (0.38 vs. 1.49 vs. 0.73), a greater proportion free from enhancing activity (12 (36, 4 %) vs. 7 (13, 2 %) vs. 6 (15 %)) and with improved EDSS (44.1 vs. 17.0 vs. 25 %).

Conclusions: The cohort design contributed to clinically important treatment group imbalances at baseline that precluded conclusive assessment of the safety and efficacy profile of abatacept in RRMS. However, while the abatacept 2 mg/kg group experienced more MS-related symptoms and relapses, data from the abatacept 10 mg/kg cohort were suggestive of reduced disease activity.

O141

FTY720 in relapsing MS: results of a double-blind placebo-controlled trial with a novel oral immunomodulator

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on behalf of the FTY720D2201 Study Group

FTY720 is an oral immunomodulator (sphingosine-1 phosphate receptor (S1P) modulator) that reversibly sequesters tissue damaging T and B cells away from blood and the central nervous system to peripheral lymph nodes. FTY720 has demonstrated both preventive and therapeutic efficacy in several animal models of MS.

Methods: We report the clinical and MRI results of an international, multicenter, double-blind study to evaluate efficacy, safety and tolerability of two doses of FTY720 and placebo (PL). 281 patients with active relapsing MS were randomized to receive PL (n = 93), 1.25 mg (n = 94) or 5.0 mg FTY720 (n = 94) q. d. for 6 months. Patients had monthly cranial MRI scans and 3-monthly neurological assessments by a neurologist otherwise not involved in their care.

Results: Clinical and MRI baseline characteristics were balanced amongst groups. The primary outcome, mean (median) total number of Gadolinium(Gd)-enhancing lesions in monthly post baseline MRI scans was 14.8 (5.0), 8.4 (1.0) and 5.7 (3.0) for PL, FTY 1.25 and 5.0 mg groups ($p < 0.001$ 1.25 vs. PL, $p = 0.006$ 5.0 vs. PL). Similar, clearly significant effects favoring both FTY720 groups vs. PL were found for Gd-enhancing lesion volume, new T2 lesions and change in T2 lesion volume (only 5 mg qd sign. better than PL). The proportion of relapse-free patients (70.0, 86.0 and 86 %; $p = 0.007$ 1.25 mg vs. PL, $p = 0.008$ 5 mg vs. PL), annualized relapse rate (0.77, 0.35 and 0.36) and time to first relapse were significantly better in both FTY720 groups vs. PL. There was no compelling dose-related difference in efficacy on MRI or clinical endpoints. Treatment was generally well tolerated with 255 (91 %) of patients completing study and 249 (89 %) electing to continue into the extension phase where PL patients were rerandomized to one of the active drug dose groups. Adverse events were more common in the 5 mg group, with the most frequently reported (> 15 % patients) being mild headaches and nasopharyngitis.

Conclusion: This proof of concept study demonstrated efficacy of FTY720 on both MRI and relapse-related endpoints. Both the efficacy and safety evaluations strongly suggest that FTY720 has the potential to be an efficacious disease modifying treatment for relapsing forms of MS with the additional benefit of once daily oral administration.

Study supported by Novartis Pharma AG Basel.

Session 23

Higher function disorders 1

O142

Interaction between CYP19-aromatase and butyrylcholinesterase genes increases Alzheimer's disease risk

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Background: Biological evidence suggests that the enzymes aromatase (CYP19) and butyrylcholinesterase (BCHE) play a role in disrupting the cholinergic neurotransmission observed in Alzheimer's disease (AD). CYP19 is a critical enzyme in the peripheral synthesis of estrogens, which

may reduce the risk of AD through enhancing cholinergic neurotransmission. BCHE is a hydrolytic enzyme associated with acetylcholine synaptic degradation, and the BCHE K genetic variant confers some protective effect for AD by reducing the activity of the enzyme.

Objective: We investigated whether a 5'-UTR CYP19 polymorphism and the BCHE K variant might be responsible for susceptibility to AD.

Methods: The study included 187 AD patients (70 % women; mean age 75.0 years; SD 8.9; range 50–97 years) who met NINCDS/ADRDA criteria for probable AD. Control subjects were 172 unrelated individuals (74 % women; mean age 79.8 years; SD 7.5; range 66–98 years) with Mini Mental State Examination scores of 28 or more, which were verified by at least one subsequent annual follow-up assessment.

Results: When the risk was considered for a single polymorphism, the presence of the CYP19 C/C genotype conferred a marginally significant increase in the risk for AD (odds ratio, OR, 1.54, $p = 0.053$), and subjects with BCHE K allele had a decreased risk for AD (OR 0.56, $p = 0.022$). In a combined analysis of the relationship of both polymorphisms and AD, it became evident that the risk associated with the CYP19 C/C genotype was confined to BCHE K allele noncarriers (OR 1.81, $p = 0.020$), whereas no effect of CYP19 alleles was seen in the subgroup of BCHE K allele carriers (OR 0.91, $p = 0.839$).

Conclusions: CYP19 and BCHE polymorphisms may interact in determining the risk for AD. Our findings suggest that carriers of both CYP19 C/C genotype and BCHE non-K variant would have greater cholinergic deficit and, consequently, increased risk of developing AD.

O143

An analysis of communication in patients with severe traumatic brain injury

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Introduction: Traumatic brain injury (TBI) is the source of severe and lasting cognitive disorders, and more especially of dysexecutive syndrome and memory impairment. In these patients, we have little information about verbal (VC) and especially non-verbal (gestual) communication (NVC).

Objectives: To analyse VC and NVC, at the secondary phase post-TBI.

Methods: Seventeen patients were evaluated 2 to 12 months after severe TBI (GCS < 8): 14 men and 3 women; mean age: 35.3 years; mean delay: 260 days. They were evaluated using the Lille Communication Test (LCT), which is composed of 3 parts: motivation to communication (salutation, attention, participation), VC (verbal comprehension, expressivity, intelligibility, informativity and pertinence, verbal feed back), and NVC (non-verbal gestual comprehension, expressivity, informativity, non-verbal feed back). They were also presented with classical neuropsychological tests of executive functions (categorical evocation, Stroop Test, Trail Making Test), gesture production, language, and the Neurobehavioural Rating Scale (Levin). Their performance was compared to that of a group of 17 matched control subjects.

Results: At the LCT, patients were significantly impaired ($p < 0.01$) for motivation to communicate, and the global scores of VC and NVC. Furthermore, the deficit was severe in each subtest evaluating motivation, and in most of those assessing VC. Conversely, those assessing NVC were relatively preserved. Subtests of motivation and VC were correlated with executive tests, while those of the NVC were not. VC subtests were also correlated with language assessment.

Conclusion: This study showed a severe reduction in the motivation to communicate and significant disorders in VC. Conversely, NVC was better preserved. It also suggested a clear relation of motivation and VC disorders with the dysexecutive syndrome, which is the consequence of predominant pre-frontal lesions.

O144

Positron emission tomography in the characterisation of mild cognitive impairment in elderly people over 70 years

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Introduction: Early diagnosis of Alzheimer's disease (AD) is still a challenge and there are no specific markers to identify elderly people with mild cognitive impairment (MCI) who will become AD patients in the future.

Objectives: To analyze the correlation between the findings on neuropsychological tests and PET-CT explorations in elderly people healthy cognoscitive subjets HC, with MCI and AD.

Methods: Observational transversal study in elderly people over 70 years. Informed consent has to be signed. Neuropsychological tests (MMSE, BDRS, IDDD, STAI, GDS, estrés appreciation, FCSRT, verbal categorial fluency test) and the 18F-FDG (PET-CT) by visual qualitative and semiquantitative appreciation by the Segmental Analysis program were done.

Statistical Analyses: U de Mann-Whitney and Fisher exact test.

Results: Out of 65 patients who were evaluated, 47 (24 women) were included in this study.

Mean age was 76 ± 5 years. Studies: 21 degrees, 26 secondary or primary.

Regarding as their cognitive stage were classified HC: $n = 30$, MCI: $n = 9$ and mild AD (NINDS-ADRDA): $n = 8$; MMSE: HC: 33 ± 4, MCI: 32 ± 1, EA: 28 ± 3 ($p < 0.05$);

BDRS: HC: 1.2 ± 3, MCI: 4 ± 5, AD: 5.8 ± 2 ($p < 0.05$); STAI: HC: 11 ± 7, MCI: 10 ± 7, AD: 21 ± 15 ($p < 0.05$); GDS: HC: 8 ± 6, MCI: 9 ± 6, AD: 11 ± 4 (NS); Stress appreciation: HC: 20 ± 10, MCI: 21 ± 11, EA: 32 ± 15 ($p < 0.05$); FCSRT: HC: 40 ± 8, MCI: 18 ± 13, AD: 17 ± 15 ($p < 0.05$); verbal categorial fluency: HC: 18 ± 10, MCI: 12 ± 5, AD: 6.8 ± 4 ($p < 0.05$); IDDD: HC: 35 ± 8, MCI: 40 ± 10, AD: 49 ± 11 ($p < 0.05$). On the PET qualitative evaluation the percentage of subjects with decrease or defects in FDG uptake were greater in the group of MCI and AD respect of the HC group ($p < 0.05$) and the Segmental Analysis program revealed statistically significant differences in segmental 18F-FDG uptake in the segments from the temporal and parietal lobes in the AD and MCI front of HC ($p < 0.05$).

Conclusions: Neuropsychological tests as PET-CT explorations can discriminate between aged people cognitively healthy and patients with MCI or mild AD.

PET together with other neuropsychological evaluations could be really interesting for these patients if larger series confirm these findings.

O145

Postoperative cognitive dysfunction

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The present study has been performed with the purpose to analyze POCD after operations with regional (RA) and general anesthesia (GA) of various duration in young patients with non-complicated psychoneurological and somatic anamnesis.

45 patients (female – 12, male – 33) with the mean age of 29.8 ± 3.1 years have been examined at 1–2 days before and at 9–10 days after the operations. The examination included neurological assessment, battery of neuropsychological testing. The RA group consisted of 21 (46.6 %) patients after Kulenkampf humeral plexus block. The GA group included 24 (53.3 %) patients after general combined intravenous anesthesia. RA was performed under the conditions of preserved consciousness and spontaneous respiration. GA was carried out under the conditions of artificial ling ventilation. During the operation stable hemodynamics was registered, mean blood loss was 120 ± 25 ml, and no signs of anesthetics overdosing were detected. After the operation under GA, all patients were extubated and transported to the ward being in consciousness with self-breathing. Duration of anesthesia was 3 hr 25 min ± 0.05 hr (range – 1 hr 30 min – 5 hrs). There was no significant difference in the anesthesia duration between groups ($p > 0.05$).

Results of testing in postoperative period comparing with the preoperative one showed no significant difference in patients of RA group ($p > 0.05$).

In the GA group the negative dynamics of such parameters has been revealed. This included appearance of complaints on gravity in the head (16.7 %), dizziness with unsteady walking (20.8 %), day-time sleepiness (29.2 %), subjective suppression of usual actions (25 %), nystagmus at side looking (41.7 %), ataxia in Romberg pose (25 %) and in flank walking (20.8 %), fluttering tremor (8.3 %) ($p < 0.05$).

Increase of mistakes number and decrease of mean working capacity coefficient (WCC) from 0.967 ± 0.02 (preoperative period) to 0.904 ± 0.10 (postoperative period) ($p < 0.05$), reduction of remembering speed and short-term memory volume were observed. Strong negative correlation between the WCC decrease and duration of the GA ($r = -0.6849$; $p < 0.01$), positive correlation between the number of words presentations and duration of GA ($r = 0.8364$; $p < 0.01$), as well as between the mistakes appeared in testing and GA duration ($r = 0.6986$; $p < 0.01$) were found.

GA makes a negative impact on cognitive functions in young patients. Expression of cognitive functions alterations depends on the GA duration.

O146
Axial subjective straight-ahead in neglect patients
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Background: Though the egocentric reference was conceived as a representation of a plane, i. e. the midsagittal plane which includes body axis, research has concentrated on its horizontal projection (horizontal straight-ahead assessment). Especially, the possibility that its axial projection is biased in neglect was not investigated.

Objective: To assess the distortions of the representation of the body axis in a sitting position, in patients with left spatial neglect.

Methods: Patients presented with a right hemisphere lesion and were either neglect (N+: 6 cases), or non neglect (N–: 6), in classical 'paper an pencil' tests. They were compared to 6 matched control subjects (C). We used a new testing device, which simultaneously assessed the lateral position (translation) and the frontal inclination (rotation) of the representation of the longitudinal axis of the head, the trunk or both as a whole. Subjects sat in the dark and had to adjust a luminous rod opposite the body part axis.

Results: In translation, N+ patients showed a severe right-sided horizontal deviation (+3.8 cm) of their 'subjective straight ahead' (SSA), which was more severe for the caudal and lower body part (trunk) than for the rostral and upper one (head). Conversely, accuracy was fair in N- patients (–0.4 cm) and C subjects (+0.4 cm). In rotation, an anti-clockwise frontal deviation was observed in both patient groups, which was more severe when neglect (–4.7° and –2.3° respectively), and absent in C subjects (+0.1°). This deviation was of similar amplitude for each body part.

Conclusions: Neglect patients present with an ipsilateral translation of the subjective straight-ahead, less severe for more rostral body parts. This distortion of the representation of the body axis is congruent with the classical data from straight-ahead studies.

O147
Very early onset of familial frontotemporal dementia linked to chromosome 17 associated with tau gene mutation g389r
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This previously healthy young woman was 17 years old when she progressively developed severe behavioral disturbances. She was first considered to suffer from a psychosis. After 2 years, she was referred to the department of neurology. By this time, the patient exhibited a clear dementia with mild extra-pyramidal disorders. Main behavioral disturbances included apathy, depressed mood and reduced speech output. Neuropsychological assessment demonstrated impaired executive functions, whereas orientation, visuo-spatial functions and memory were relatively preserved. MRI and SPECT scan showed respectively frontotemporal atrophy and hypometabolism within the same regions. We discovered that 3 relatives on her father's side died after an early onset dementia: her uncle at 36, her grandfather at 46, her great-grand-father at 42. Five years post onset, the patient is bedridden, with extra-pyramidal rigidity, dysphagia, bladder and bowel incontinence.

The sequencing of Tau gene revealed the mutation G389R, on exon 13, codon 389 (transcription C-G). This case is remarkable because of the extremely early onset, contrasting with the normal state of the patient's father who is 43 and presents the same mutation. We discuss the mechanism of a possible anticipation.

Session 24

Higher function disorders 2

O148
Dorsolateral frontal cortex activation may reflect action shifts in a tool use pantomime task: a functional magnetic resonance imaging (fMRI) study
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Background and Objectives: Previous fMRI studies have shown that the left intraparietal cortex (LIPC) and the left dorsolateral cortex (LDLC) may be activated during ideomotor praxis tasks. However, it remains unclear how far these regions are linked to tool-use or to action-shift demands. We intended to assess these issues by a tool-use pantomime fMRI protocol using two different task-subtraction conditions varying in their action-shift load.

Methods: Seven healthy right-handed adults were trained to perform separately right and left hand motor sequences in three experimental conditions: 1: 24 types of tool-use pantomimes; 2: a single multistage movement repeated in a closed-loop; and 3: four distinct sequences of meaningless finger tapping movements. Each patient was then submitted to 8 alternating periods of these 3 conditions for fMRI acquisition, totalizing 24 periods of data collection per hand. In tool-use pantomime periods, the name of 3 among the 24 learned tools were indicated in sequence (e. g.: brush, scissors, screwdriver). In the multistage sequence task, the name of 3 non-tool objects (e. g.: tree, window, sun) were also spoken in sequence in order to match for auditory stimulus. In the finger tapping movements, 3 of the 4 learned sequences were randomly spoken to cue the motor sequences. Blood oxigenation level dependent (BOLD) increases were subtracted between conditions 1 and 2 as well as 1 and 3. Functional and anatomic data were then overlaid.

Results: The left hemisphere was significantly more activated than the right one regardless of the hand used. Activation clustered in the immediacy of the LIPC when either the second or third experimental conditions were subtracted from the first one. However, activation clustered in the LDLC only when the second experimental condition was subtracted from the first one. Seemingly, the subtraction of the third from the first condition showed no consistent activation in LDLC.

Conclusions: 1.The activation of the LIPC during tool use pantomime and regardless of action shift load reinforces its importance in ideomotor praxis. 2. The preferential activation of the left hemisphere regardless of the hand used suggests that ideomotor praxis is a lateralized function. 3. The absence of DLFC activation when switching finger tapping sequences were subtracted from switching tool-use pantomimes indicates a potential role in action shift monitoring.

O149
Influence of body positioning on the subjective visual vertical in neglect patients
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Background: An anti-clockwise (ACW) deviation of the subjective visual vertical (SVV) is known to occur in patients with right hemisphere lesion and spatial neglect. Though postural changes proved to influence other signs of neglect, SVV has only been assessed when sitting.

Objective: To assess the influence of progressive postural changes on SVV deviation.

Methods: Eight patients presenting with a right hemisphere lesion and spatial neglect (N) in classical 'paper an pencil' tests were included and compared to 4 matched control subjects (C). They had to mobilise a luminous rod in the dark, in rotation, in order to put it at the vertical, in 4 conditions: (1) sitting with plantar sole support; (2) sitting without plantar sole support; (3) sitting with legs extended on a support; (4) supine position. We measured the deviation of the rod from the objective vertical.

Results: Neglect patients showed a significant ACW deviation (–4.5°) of the SVV, as compared with C subjects, which were perfectly normal (+0.01°). The main effect of body position was not significant ($p = 0.127$), but this factor interacted with the group ($p = 0.022$), as changes in positioning had definite effects in the N, but not in the C. In fact, the former showed a progressive and regular reduction of the CCW deviation, from the first to the fourth condition, the supine condition differing significantly from the others.

Conclusions: While the possible role of plantar and leg somaesthetic inputs remains to be confirmed, the modulation of gravitational inputs at trunk and/or vestibular level clearly influences the SVV deviation in neglect patients. This has to be put in relation with the modulation of neglect signs reported by other authors, especially the ipsilateral deviation in bisection tasks when passing from the sitting to the supine position.

O150

Interaction between inflammatory genes is associated with Alzheimer's disease risk

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Background: Genes encoding cytokines (interleukins-IL-1A, 6, and 10), chemokines (IL-8), and intercellular adhesion molecules (ICAM-1) might contribute to the neurodegeneration associated with Alzheimer's disease (AD), by provoking a chronic inflammatory response in the brain.

Objective: We investigated whether the combined genetic effects between IL-1A (–889), IL-6 (–174), IL-8 (–251), IL-10 (–1082), and ICAM-1 (E469K) polymorphisms might be responsible for susceptibility to AD.

Methods: The study included 276 AD patients (69% women; mean age 75.6 years; SD 8.9; range 50–98 years) who met NINCDS/ADRDA criteria for probable AD. Control subjects were 237 unrelated individuals (71% women; mean age 79.9 years; SD 7.9; range 63–98 years) with Mini Mental State Examination scores of 28 or more, which were verified by at least one subsequent annual follow-up assessment.

Results: The presence of the IL-1A allele 2 conferred a marginally significant increase in the risk for AD (odds ratio, OR, 1.42, $p = 0.053$), and the presence of the IL-8 T/T genotype was not associated with AD (OR 1.33, $p = 0.135$), but the presence of both IL-1A allele 2 and IL-8 T/T genotype was associated with a twofold increased risk for AD (OR 2.12, $p = 0.007$). The presence of the IL-6 C/C genotype conferred a significant twofold decreased risk for AD (OR 0.55, $p = 0.034$), the presence of the IL-10 A/A genotype was not associated with AD (OR 0.71, $p = 0.102$), and the presence of the ICAM-1 K/K genotype was not associated with AD (OR 1.08, $p = 0.732$); however, the subjects carrying both the IL-6 C/C and the IL-10 A/A genotypes had about a five times lower risk of developing AD (OR 0.18, $p = 0.005$), and the subjects carrying both the IL-6 C/C and the ICAM-1 K/K genotypes had about a four times lower risk of AD (OR 0.26, $p = 0.046$).

Conclusions: Gene-gene interaction between IL-1A and IL-8 increases AD risk and interaction between IL-6 and either IL-10 or ICAM-1 decreases AD risk. Considering synergistic effects between polymorphisms in genes encoding inflammatory mediators may help in determining the risk profile for AD.

O151

A longitudinal MTR study in mild to severe Alzheimer's disease

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Background: The magnetization transfer ratio (MTR) is widely used as a sensitive measure to probe structural changes of the brain tissue. Recent MTR analyses in patients suffering from Alzheimer's disease (AD) suggest that pathology in AD is global and that a whole brain MTR histogram analysis might serve as surrogate marker for the overall brain damage. This study aimed at investigating the temporal evolution of global MTR parameters in AD patients and to study their association with changes in neuropsychological performance.

Material and Methods: 21 patients (age 61–87 years) diagnosed with AD underwent serial MRI and cognitive testing including the Mini-Mental State Examination (MMSE), clinical dementia rating (CDR), and the Alzheimer's Disease Assessment Scale (ADAS-COG). MRI was performed on a 1.5T whole body scanner and included conventional imaging and MT imaging. The MT sequence was based on a spoiled 2D gradient echo that was performed with and without a binomial MT saturation pulse. A follow-up MRI was performed after 6 months and 11 patients had an additional MRI after 12 months. Using a brain extraction tool, brain masks were produced for the baseline scans to identify those pixels that were included in the histogram analysis. These masks were then used for all follow-up MTR maps after they had been registered to the baseline examination. The following parameters were derived from the histograms: mean MTR value, relative peak height, and peak position.

Results: All histogram parameters showed a significant decrease over time. The most marked changes were found for the mean MTR ($p < 0.01$) and the peak position ($p < 0.001$). The correlation between changes of specific histogram parameters and in neuropsychological performance was poor or absent. However, regression analysis revealed that the peak position was a predictor for subsequent changes of the neuropsychological performance. We found significant correlations between the MTR peak position and subsequent changes in the MMSE ($R = 0.55$, $p = 0.001$) and ADAS ($R = –0.5$, $p = 0.003$) 6 months later.

Conclusion: Our results indicate a very high sensitivity of MTR histogram metrics for ongoing tissue changes in AD as we observed a significant decline of all MTR histogram metrics already within 6 months with a relatively small sample of AD patients. The fact that MTR metrics were predictive of cognitive deterioration will have to be further explored.

O152

Experimental autoimmune tauopathy: immunoreactivity against tau-microtubule-associated protein induces a neurological disease in mice

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Objective: To investigate whether immunization with tau microtubule associated protein induces a neurological disease in animals. The finding may point to a role that autoimmunity against the tau protein might play in neurological disorders, including neurodegeneration.

Background: A possible role of autoimmunity in the pathogenesis of Alzheimer's disease (AD) has lately attracted a great attention. Naturally occurring antibodies (Abs) against the beta-amyloid (A) have been reported. Moreover, immunization with A attenuates amyloid pathology, but also induces neuroinflammation. Very little is known about the other major component of AD pathology – tau aggregation into neurofibrillary tangles (NT). We recently reported the presence of naturally occurring anti-tau Abs targeted against unphosphorylated and also AD-related phosphorylated tau in AD and controls, with higher IgM anti-phosphorylated tau in AD. These results may point to a possible involvement of immune mediated processes against the tau protein. To investigate this possibility we actively immunized healthy mice with tau protein and tested whether a neurological disease is induced.

Design/Methods: We immunized C57BL female mice with recombinant human tau protein emulsified in complete Freund's adjuvant (CFA).

Results: A neurological deficit was detected in 5 of 12 immunized animals in two successive experiments, presenting a limp tail (one of them also with leg paralysis). None of the CFA control mice ever showed any signs of disease. A significant increase in anti-tau Abs was detected in the tau immunized animals. Preliminary results demonstrate monocyte infiltrates in brain sections. The brains and spinal cords are currently under investigation, for the presence of any neuropathological or possible neurodegenerative changes.

Conclusions: In this study we demonstrated a neurological deficit induced by immunization with the tau microtubule associated protein. To our best knowledge this is the first report of a neuropathological effect related to anti-tau immunoreactivity; and moreover, no previous description of a neurological deficit induced by a neuronal protein was reported so far. These results may shed light on the potential role that autoimmune mediated processes against neuronal proteins may play in neurological/neurodegenerative diseases, and may open opportunities for therapeutic approaches.

O153

PS1 P284S mutation associated with dementia, pyramidal system involvement and multiple sclerosis-like MRI abnormalities

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Introduction: Familial Alzheimer's disease (FAD) is an autosomal dominant genetically heterogeneous entity. Although mutations in the amyloid precursor protein (APP) gene and in presenilin 2 are causative of several FAD cases, the majority of patients are carriers of presenilin 1 (PS1) gene alterations.

Individuals affected by FAD phenotypically do not differ from to the sporadic Alzheimer's disease, except for earlier age at onset. However, several subjects with FAD exhibit a peculiar clinical phenotype characterized by presenile dementia associated with spastic paraparesis. In addition, most of them showed the distinct neuropathological finding of "cotton wool" plaques, devoid of congophilic dense core and plaque-related neuritic pathology.

Patients and methods: We studied a family consisting of an affected fa-

ther (I-2), healthy mother (I-1) and three sons, two clinically affected (II-1, II-2) and an asymptomatic one (II-3). Neurological, neuropsychological examinations and brain MRI were performed in all family members. Screening for mutations in the PS1 gene was carried out by PCR and subsequently sequencing reactions. Ten coding exons 3–12 of the PS1 gene were amplified separately using primers designed to the flanking intronic sequences.

Results: Sequence analysis of the entire coding region of the PS1 gene showed a heterozygous point mutation C1097 T in subjects I:1, II:1, II:2 and II:3 which results in the replacement of the amino acid proline to serine (P284S) in the exon 8. Affected subjects showed evidence of early-onset dementia ranging from 32 to 45 years, pyramidal system involvement which included spastic para-tetraparesis, dysarthria and dysphagia and MR white matter T2-weighted hyperintense lesions meeting the criteria for multiple sclerosis. White matter alterations predated cognitive dysfunction and pyramidal signs, since they were observed in one pre-symptomatic subject, while in advanced stage of the disease, the hyperintense lesions involved the whole brain white matter.

Conclusions: The P284S mutation, located in the PS1 T6 domain, is likely to be responsible for this novel variant of FAD. Moreover, we underline the possibility that some subjects with MR white matter lesions consistent with a diagnosis of MS who had other relatives affected with dementia should be considered for alternative diagnosis, as demonstrated by subjects presented in this report.

Session 25

Multiple sclerosis 5

O154

A comparative study of interferon-beta products for relapsing-remitting multiple sclerosis: QUASIMS results from Italy

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Interferon beta (IFNB) is widely used as first-line treatment for relapsing remitting multiple sclerosis (RRMS). Clinical trials have demonstrated that IFNB reduces relapse rate (RR), increases the proportion of relapse-free patients (pts), and increases the time to disease progression by approximately one-third. Quality Assessment In MS therapy (QUASIMS) is a retrospective, multinational, chart review study initiated in German-speaking countries to compare efficacy and tolerability of the four available IFNB preparations in pts with RRMS. An adapted QUASIMS questionnaire, including data on pre-treatment pt characteristics, was used in Italy. 898 consecutive RRMS pts received uninterrupted treatment with intramuscular IFNB-1a 30 micrograms (mcg) once weekly (A), subcutaneous (sc) IFNB-1b 250 mcg every other day (B), sc IFNB-1a 22 mcg three times a week (C), or sc IFNB-1a 44 mcg three times a week (D) for at least 2 years. Patients who received one or more disease-modifying treatments for less than 2 years, then received one of the 4 IFN betas uninterrupted for 2 years are referred to as the follow-up group. Preplanned outcome measures included change from baseline EDSS over 2 years and percentage (%) of relapse-free pts. Differences among treatment groups were analyzed using a regression model (covariates: age at treatment initiation, baseline EDSS score, disease duration). The 4 groups of pts (39 % treated with A, 20.7 % with B, 25.1 % with C and 15.3 % with D) were comparable for age, disease duration, EDSS and RR in the 2 years before treatment, although the % of males was slightly higher in B and patients were somewhat younger and had a higher RR in D. At 1 year, the change from baseline EDSS was – 0.12 (A), –0.06 (B), –0.16 (C), and –0.11 (D) and at 2 years, the change from baseline EDSS was – 0.07 (A), +0.09 (B), –0.07 (C), and –0.14 (D). The % of relapse-free pts at 2 years was 54.6 (A), 54.3 (B), 51.6 (C), and 48.9 (D). In the follow-up group the % of relapse-free pts at 2 years was 50.0 (A), 66.7 (B), 40.0 (C), and 53.6 (D). No significant differences in any of the efficacy outcome measures were observed among the 4 groups. In the follow-up group, switching to a higher dose or more frequent administration provided no additional benefit in terms of relapse rate. QUASIMS results from Italy confirm that in the common clinical setting in pts continuing treatment for at least 2 years IFNB products have comparable efficacy and tolerability profiles.

O155

QUASIMS comparative study of interferon-beta products for relapsing multiple sclerosis: results from Belgium, Netherlands, and Luxembourg

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Interferon (IFN)-beta products have been first-line treatment options for relapsing multiple sclerosis (MS) since their approval. Comparative studies have shown that IFN-beta therapies have similar clinical efficacy. QUASIMS BeNeLux was conducted to compare the efficacy of IFN-beta preparations as disease-modifying therapies for relapsing MS in Belgium and the Netherlands. QUASIMS is a retrospective, controlled, open-label, international, observational study. It is the largest IFN-beta comparative trial with complete patient records from over 7000 patients with relapsing MS in several countries. Patients with a diagnosis of relapsing MS and at least 2 continuous years of treatment with: (A) intramuscular IFN-beta-1a 30 mcg once weekly, (B) subcutaneous IFN-beta-1b 250 mcg every other day, (R22) subcutaneous IFN-beta-1a 22 mcg three times a week, or (R44) subcutaneous IFN-beta-1a 44 mcg three times a week were included. Patients who received one or more disease-modifying treatments for less than 2 years, then received one of the 4 IFN betas uninterrupted for 2 years are referred to as the follow-up group in all data analyses. Outcomes included change from baseline EDSS, and the proportion of relapse-free patients at 2 years. Differences among treatment groups were analyzed using a logistic regression model (covariates: age at treatment initiation, baseline EDSS score, disease duration). Of the 508 patients evaluated, 43.7 % were treated with A, 34.4 % were treated with B, 15.9 % were treated with R22 and 5.9 % were treated with R44. At baseline, patients had a mean age of 39.6 years and 64 % were female. At one year, the change from baseline in EDSS was 0.11, 0.05, 0.07, and 0.08 in the A, B, R22, and R44 groups, respectively. At 2 years, the change from baseline EDSS was 0.24 (A), 0.27 (B), 0.25 (R22), and 0.17 (R44). The proportion of relapse-free patients at 2 years was 51.4 % (A), 41.7 % (B), 38.3 % (R22), and 46.7 % (R44). In the follow up group, the percentage of relapse-free patients at 2 years was 26.5 (A), 16.7 (B), 11.1 (C), and 18.8 (D). No significant differences in any of the efficacy outcomes between treatments were observed and in the follow up group, switching to a higher dose or more frequent administration provided no additional benefit in terms of relapse rate. In Belgium and the Netherlands, similar clinical efficacy was observed with 2 years of treatment with each of the interferon-beta products in patients with relapsing MS.

O156

The OPTIMS study. I. Looking for a treatment response marker

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Objective: To evaluate whether an active MRI scan during the first 6 months of IFN beta treatment for relapsing-remitting (RR) MS is a reliable indicator of both persisting disease activity in spite of immunomodulatory drug treatment over a 2-year follow-up period.

Background: The OPTimization of Interferon (IFN) for MS (OPTIMS) trial is a multicenter trial designed to find a reliable indicator of suboptimal treatment response to IFN beta-1b and to test the efficacy of 375 mcg IFN beta-1b.

Design/methods: During a 6-month run-in phase, all patients were treated with the approved IFN beta-1b dose (250 mcg, subcuteaneous (SC), every other day) (EOD). With monthly MRI scans, suboptimally responding patients (those with at least a new T2 or gadolinium-enhancing lesion) were randomized to continue the same IFN beta-1b dose or to increase the dose to 375 mcg, SC EOD. Patients were followed up for a further 6 months with monthly MRI scans, and for a further year with yearly scans. The primary outcome measure was the occurrence of further clinical disease activity (relapses or confirmed EDSS progression). ROC analysis was used to assess sensitivity, specificity, and predictive power of one or more active scans during first 6 months.

Results: From 216 enrolled patients, 40 patients with a suboptimal treatment response were randomized to 250 mcg, and 36 to 375 mcg. One-hundred-and-four patients resulted to be good responders to treatment (no MRI activity, no relapses) and continue on 250 mcg EOD for 2 years. Thirty-six patients were withdrawn from treatment during the first 6 months of the trial. In order to simulate clinical practice as closely as possible, only data from patients continuously treated with 250 mcg IFN beta-1b were analyzed. A single active scan after 6 months of treatment identified patients with persisting disease activity with a high sensitivity and predictivity power. Backward stepwise regression logistic analysis indi-

cated that this was the most reliable indicator compared to all other active scan combinations (P = 0.03).

Conclusion: Over a 2 year follow-up, patients with an active scan after 6 months of IFN beta-1b treatment, had persisting clinical disease activity in spite of the immunomodulatory drug treatment at the approved dose. Those patients could be considered for an adjustment in treatment.

O157

Defining beta-interferon failure in multiple sclerosis: impaired relapse rate reduction predicts progressive disability

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Objective: To investigate whether the relapse rate response to beta-interferon (IFNB) is related to the development of sustained disability in the treatment of relapsing-remitting multiple sclerosis (RRMS).

Methods: In this study of 177 RRMS patients treated with IFNB, patients were followed at 6 to 12 monthly intervals for up to 5 years. Relapses were noted at each assessment and a Kurtzke Expanded Disability Status Scale (EDSS) measurement was performed at the start of IFNB therapy and at least yearly thereafter. Progressive disability was defined as a one-point increase in the pre-treatment EDSS sustained over six months. IFNB failure was defined as (1) no reduction in the individualised 24-month pre-treatment relapse rate and/or (2) two disabling relapses in a twelve-month period of IFNB therapy (Association of British Neurologists criterion for IFNB failure). Patients not meeting IFNB failure criteria and who did not develop progressive disability were deemed to be IFNB responders.

Results: Of the 177 patients, 83 (46 %) were deemed to be IFNB responders and 94 (54 %) met IFNB failure criteria; 60 (63 %) of the patients meeting IFNB failure criteria developed progressive disability. The mean relapse rate in the first year of IFNB therapy for the IFNB responders group was 0.13 (95 % CI: 0.05–0.20) and in patients who developed progressive disability 0.46 (95 % CI: 0.29–0.64) (p = 0.01); the relapse rates in the second year of IFNB therapy were 0.16 (95 % CI: 0.07–0.25) and 0.43 (95 % CI: 0.27–0.59) (p = 0.02) respectively.

Conclusion: Patients without a reduction in their 24-month pre-IFNB relapse rate or who develop two disabling relapses during the first two years of IFNB therapy should be regarded as being at high risk for the subsequent development of progressive disability.

O158

The effect of oral temsirolimus on new magnetic resonance imaging scan lesions, brain atrophy, and the number of relapses in multiple sclerosis: results from a randomised, controlled clinical trial

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Background: Temsirolimus is being developed as an oral, disease-modifying treatment for multiple sclerosis (MS). It is active in experimental autoimmune encephalomyelitis and selectively blocks interleukin 2-driven T-cell proliferation. The efficacy and safety of temsirolimus were evaluated in patients with clinically definite relapsing-remitting MS (RRMS) or secondary progressive MS with relapses.

Methods: A multicenter, randomized, double-blind, placebo-controlled, phase 2 clinical trial was conducted in 296 patients aged 19–57 years. Eligibility criteria included baseline Expanded Disability Status Scale (EDSS) scores of 0–6.5 and ≥1 documented relapse in the preceding 12 months before screening or in the preceding 24 months before screening with at least 1 enhancing T1 lesion on screening or baseline magnetic resonance imaging (MRI) scan. After random assignment, patients received oral temsirolimus (2, 4, or 8 mg 1x daily) or placebo for 9 months. MRI scans were performed at screening, baseline, and monthly. The primary endpoint was the cumulative number of new Gd-enhanced T1 lesions at 9 months on MRI. Total brain volume, number of relapses, mean EDSS scores, other MRI measures, and health outcomes were secondary endpoints.

Results: Patient demography was similar among treatment groups. Most patients (85 %) had RRMS; the median EDSS score was 2.5. Patients receiving 8-mg temsirolimus achieved significant reductions (47.8 %) in the cumulative number of new Gd-enhancing T1 lesions on MRI compared with placebo (p = 0.010). MRI endpoints showed a dose response, the 8-mg dose reaching statistical significance for the primary endpoint by 32 weeks (p = 0.024). Brain volume data suggested a decrease in brain volume atrophy at 36 weeks in the 8-mg group compared with placebo. The 8-mg group showed a 51 % reduction in number of relapses per patient vs. placebo (p = 0.023). Dose-related trends in percentage of relapse-free patients and progression of disability were also noted. Serious adverse events occurred at similar frequencies across all treatment groups. Aphthous stomatitis/mouth ulceration, hyperlipidemia, rashes, and menstrual dysfunction were reported more often in the 8-mg group vs. placebo.

Conclusions: An oral, 8-mg dose of temsirolimus administered over 9 months in patients with relapsing forms of MS resulted in significant beneficial effects on the incidence of new enhancing MRI lesions and number of relapses, with an acceptable risk/benefit profile.

O159

The efficacy of natalizumab in patients with relapsing multiple sclerosis: subgroup analyses of AFFIRM

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For the AFFIRM Investigators

Objective: To determine the efficacy of natalizumab (TYSABRI®) in prespecified subgroups of patients in AFFIRM.

Background: Natalizumab is the first alpha4-integrin antagonist in the new class of selective adhesion molecule (SAM) inhibitors for the treatment of MS. The AFFIRM study showed that natalizumab significantly reduces clinical relapses, attenuates MRI lesion formation, and is safe and well tolerated in patients with relapsing MS. Further analyses were conducted in pre-specified subgroups of AFFIRM in order to evaluate the efficacy of natalizumab in patients with more or less active inflammatory disease.

Study Design: AFFIRM is a randomized, double-blind, placebo-controlled, multicenter phase III trial of natalizumab in patients with relapsing MS. Patients were randomized to receive natalizumab 300 mg or placebo intravenously every 4 weeks for up to 116 weeks. The primary endpoints at 1 and 2 years were the rate of clinical relapses and disability progression as measured by the Expanded Disability Status Scale (EDSS), respectively. The effects of natalizumab on primary endpoints were analyzed on the following pre-specified patient subgroups based on their baseline characteristics: pre-study relapse rate (1, 2, > 2), EDSS score (≤3.5, > 3.5), number of T2 lesions (< 9, ≥9), presence of gadolinium-enhanced (Gd+) lesions (0, ≥1), age (< 40, ≥40), and gender (male, female).

Results: Natalizumab significantly reduced relapse rate compared with placebo in all pre-specified subgroups after a median of 13 months of treatment, except the small cohort of patients with < 9 T2 lesions that had a low annualized relapse rate irrespective of treatment group. For example, the annualized relapse rate at 1 year was 0.23 natalizumab vs. 0.66 placebo, 0.32 natalizumab vs. 0.91 placebo, and 0.27 natalizumab vs. 1.45 placebo in patients with 1, 2, or > 2 relapses at baseline, respectively. In general, examination of the placebo group showed that patients with more active inflammatory disease at baseline (as measured by a higher number of T2 lesions, the presence of Gd+ lesions, and more relapses) developed more lesions and had more relapses on study compared with patients with less active inflammatory disease activity. Two-year data will be presented.

Conclusions: Natalizumab reduces clinical relapses in patients with relapsing MS regardless of demographic characteristics or disease severity.

O160

Magnetic resonance imaging results from AFFIRM: a randomised controlled trial of natalizumab in patients with relapsing multiple sclerosis

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For the AFFIRM Investigators

Objective: To determine the effect of natalizumab (TYSABRI®) on lesion formation as measured by magnetic resonance imaging (MRI) over 2 years of treatment in patients with relapsing multiple sclerosis (MS).

Background: Natalizumab, the first selective alpha4-integrin antagonist for the treatment of MS, inhibits the migration of leukocytes across the blood-brain barrier and interrupts the inflammatory cascade in MS. Natalizumab was recently approved for the treatment of relapsing MS based on 1-year results from two phase III studies, AFFIRM and SENTINEL.

Study Design: AFFIRM is a randomized, double-blind, placebo-controlled phase III trial of natalizumab in patients with relapsing MS. Patients received natalizumab 300 mg or placebo by intravenous infusion every 4 weeks for up to 116 weeks. MRI efficacy endpoints at 1 year were the number of new or enlarging T2-hyperintense lesions and the number

of gadolinium-enhancing (Gd+) lesions. MRI efficacy endpoints at 2 years were the number of new or enlarging T2-hyperintense lesions, the number of Gd+ lesions, Gd+ lesion volume, T2 lesion volume, and the number and volume of T1-hypointense lesions.

Results: A total of 942 patients were randomized to treatment with natalizumab (n = 627) or placebo (n = 315). After 1 year of treatment, natalizumab reduced the mean number of new or enlarging T2-hyperintense lesions by 80 % (1.2 natalizumab vs. 6.1 placebo; $P < 0.0001$) and the mean number of Gd+ lesions by 92 % (0.1 natalizumab vs. 1.2 placebo; $P < 0.0001$). Sixty percent of patients in the natalizumab group developed no new or enlarging T2-hyperintense lesions compared with 22 % of patients in the placebo group. In addition, 96 % of natalizumab patients had no Gd+ lesions compared with 68 % of placebo patients. The percentage of disease-free patients (defined as no relapses, no new or enlarging T2-hyperintense lesions, and no Gd+ lesions) was 46 % in the natalizumab group and 14 % in the placebo group. The effects of natalizumab on 2-year MRI endpoints will also be presented.

Conclusions: One-year results showed that natalizumab significantly reduces lesion formation in patients with relapsing MS. Two-year results will further define the effects of natalizumab on inflammatory activity and burden of disease as measured by MRI.

Session 26

Peripheral neuropathy

O161

A practical definition of conduction block in IvIg responsive multifocal motor neuropathy

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Multifocal motor neuropathy with conduction block (MMN) is treatable with intravenous immunoglobulin (IvIg) but can be mistaken for motor neurone disease or other lower motor neurone syndromes. The formal electrophysiological criteria for conduction block (CB) are so stringent that substantial numbers of patients with MMN may remain undiagnosed and miss out on appropriate treatment. Electrophysiological data were collected from 10 healthy volunteers and compared to data from 10 patients who satisfied the clinical criteria for MMN and who responded to IvIg. This produced a definition of conduction block in MMN patients which was compared with existing definitions to assess "miss rates".

Mean values for compound muscle action potential (CMAP) area, amplitude and duration were calculated in normal subjects. Results beyond 3 SD of their respective means were considered abnormal. Using these criteria, conduction block in the context of MMN was defined as a reduction in negative peak area > 25 % along a distal nerve segment or > 30 % across the proximal segment; or reduction in amplitude > 32 % across the distal segment or > 35 % across the proximal segment. All IvIg responsive patients had at least one nerve segment showing such conduction block. Over one-third of CBs occur proximally.

In the clinical setting of suspected MMN, less stringent criteria for conduction block can improve the diagnosis of this treatable disorder. Utilising some criteria from the literature would have denied treatment to over 30 % of responsive patients. Exclusions on grounds of temporal dispersion may be over-restrictive.

O162

Painful proximal lower limb diabetic neuropathy

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Objective: To evaluate the clinical, electrophysiological and neuropathologic findings in patients with painful proximal lower limb diabetic neuropathy (PPDN).

Background: Besides the common distal sensory polyneuropathy, diabetic patients may develop sensory or sensorimotor multifocal deficits due to roots, plexus and nerve trunk involvement, a radiculoplexopathy, or radiculoplexoneuropathy. There are few very well studied series necessary to learn more about this modality of diabetic neuropathy.

Design/method. We prospectively studied 32 consecutive type 2 diabetic patients most presenting a PPDN, referred between 1995 and 2003. Other causes of proximal lower limb neuropathy were appropriately ruled out. All the patients underwent an electrophysiological study performed according to standard techniques. Superficial sensory nerve biopsy of the intermediate cutaneous nerve of the thigh was performed in six patients with clinically defined PPDN in which there was no consistent needle electrodiagnostic of root and/or plexus involvement.

Results: There were 17 men and 15 diabetic type 2 women with a mean age of 65.3 years (ranging from 39 to 87 years). Relapsing course was verified in 4 patients. The proximal neuropathy was asymmetric in the majority being unilateral in six patients, and clinically evident in the right side in almost all. Proximal unilateral or bilateral motor deficit was seen in 26 patients, but mild to moderate amyotrophy of the thigh was seen in 14. Electrophysiological test showed an axonal plexoneuropathy in all cases, but in six it was inconsistent with proximal involvement. In these patients nerve biopsy showed asymmetrical axonal lesions in all specimens, but epineurial perivascular mononuclear cell infiltrates were present in three, and invading blood vessel wall (microvasculites) in one. Treatments with immunesuppressors or immunomodulator did not change the natural history. The neuropathy improved spontaneously in 6–9 months in most cases. Gabapentin and/or amytryptiline were effective for pain relief.

Conclusion: Nerve biopsy of the intermediate cutaneous nerve of the thigh should be indicated exclusively in diabetic patients when electrophysiological tests are not consistent with a proximal nerve involvement. The efficacy of immunotherapy for PPDN is questionable. It could work only as an adjuvant for pain relief treatment with consequent improvement in motor performance.

O163

Different mechanisms of sudomotor and vasodilator axon reflexes in human skin

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The neurotransmitter acetylcholine (ACh) activates sudomotor fibers as well as primary afferent nociceptors. This induces sudomotor resp. vasodilator axon reflexes, which are diminished e. g. in neuropathies. Some neuropathic pains like CRPS, however, are accompanied by increased axon reflex sweating. The aim of our study was to analyse axon reflexes in order to detect possible mechanisms of amplification. In a first step we analysed the somatotopic organisation of the sudomotor axon reflexes on the leg to detect the most sensitive area for further testing. Therefore sudomotor axon reflex areas on the foot, lower and upper leg were compared. This was done in 15 healthy young men (mean age: 25; range 24–27 years). Vasodilator flare was measured on the lower leg in 10 healthy young men (mean age: 28; 27–33 years). In both experiments the flare reaction was elicited by constant current iontophoresis (300 mC) of acetylcholine. The sudomotor axon reflex was visualized with iodine starch staining and the sweat response was quantified with capacitance hygrometry (QSART). The vasodilator flare was visualized and quantified by laser Doppler imaging. All measurements were performed during and for 10 minutes after finishing the iontophoresis. Wilcoxon's matched pairs test was used for statistical analysis.

The sudomotor axon reflex area on the lower leg increased from 30.6 cm^2 at the end of the iontophoresis to 39.2 cm^2 ($p < 0.001$) 10 minutes later, while QSART response had already decreased. In contrast, flare size and flare intensity remained nearly constant during the observation period resp. the vasodilator flare size even decreased during the ten-minute observation period from 8.1 cm^2 to 7.1 cm^2 ($p = 0.4$). This increase of sudomotor area size was identical on the foot and the lower leg (12.5 vs. 27.9, resp. 12.2 vs. 16, $p < 0.001$ for both areas). However, the sizes of the reflex areas differed, being biggest for the lower leg, followed by the foot ($p < 0.05$) and the upper leg ($p < 0.001$). The difference between foot and upper leg did not reach significance.

Although both axon reflexes share common pathways (peripheral C-fibers), their mechanisms differ. Despite the fast cleavage of acetylcholine by cholinesterases, sudomotor axon reflexes spread in the skin, indicating a possible peripheral amplification of sweating, in particular in neuropathic pains. Such a mechanism was never observed for vasodilator axon reflexes.

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O164

HLA typing in dysimmune neuropathies

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Background: A candidate group of genes possibly involved in pathogenesis of dysimmune neuropathies (DN) are the genes that code for histocompatibility molecules (HLA). 64 patients were evaluated in order to find any possible relationship between HLA alleles and anti-peripheral nerve antibodies (PNabs), found in some DN Demographic data, clinical course, neurophysiological measures along with seropositivity for PNabs and HLA typing were investigated.

Methods: The study group (SG) included 64 neuropathic patients enrolled in 2002–2004 period, excluding those with hereditary, metabolic, infective, neoplastic, autoimmune diseases and toxic causes. Control group (CG) included 49 age-matched subjects without clinical evidence of neuropathy. Clinical presentations, onset of disease, disease duration, neurophysiological analyses were evaluated in the SG. Sera were assayed for antibodies to sulfatide, GM1, asialo GM1, GQ1b and MAG. HLA typing of class I and class II loci was performed for both groups (SSP-PCR method). Statistical comparison was performed by means of Chi-Square analyses and Fisher exact test, when appropriate.

Results: Mean age of SG was 67 years, 75 % were male, 76 % patients had a sensitive-motor, 11 % motor and 13 % sensitive neuropathy. In 35 % of SG a demyelinating form was documented, in 33 % an axonal and in 32 % a mixed form. PNabs were documented in 62 % of SG, the commonest were sulfatide (28 %) and asialo antibodies (28 %). Disease duration was not significantly different between seropositive and seronegative patients.Frequency of HLA class I and class II alleles distribution was not statistically different between SG and CG. According to the presence/absence of PNabs a significantly different distribution of HLA-DRB alleles was found for patients positive for anti-GM1IgM ($p = 0.004$) and anti-GM1IgG ($p = 0.006$). As well as HLA-A32 and HLA-CW09 alleles was found more frequent in anti-sulfatides ($p = 0.008$; $p = 0.008$) and anti-MAG ($p = 0.001 p = 0.02$) positive patients.

Conclusion: Our findings have showed a different distribution of HLA class I and II alleles for specific seropositive patients, affected with DN. This could suggest that the carriership of certain HLA alleles can modulate the affinity for specific nerve epitopes. If our data are confirmed by larger studies a multifactorial hypothesis for DN could be put forward: HLA class I and class II alleles might act in triggering or sustaining an aberrant immune response against nerve epitopes.

O165

Italian multicentre study on quality of life and disability in patients with polyneuropathy

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Objectives: Neuropathy can severely deteriorate patients' quality of life and cause disability because it modifies patient's perception of the external environment and motor efficiency. The aim of the study was to evaluate quality of life (QoL), disability and clinical features of a wide sample of patients who had a diagnosis of, or who were suspected to have polyneuropathy (POLY) or mononeuropathy multiplex (MULTI). The patients had been referred to specialised centres for assessment of peripheral nervous system diseases.

Materials and Methods: We performed a large, multi-perspective and multi-measurement assessment through validated clinical measurements of disability and quality of life.

Results: The QoL picture was highly deteriorated in the enrolled sample with respect to the Italian normative sample. All age brackets were highly impaired except for older patients for whom the QoL picture was mildly impaired (with respect to Italian Norms). With regard to general disability, more than 1/10 of patients needed assistance to walk. With regard to the arm disability, about 10 % of patients had severe disability (impairment in carrying out simple tasks). General disability picture was similar both in the polyneuropathic and multineuropathic groups but the arm disability appeared more severe in multineuropathic patients. Thirty-two percent of patients with POLY had concomitant diabetes (68 % had no concomitant diabetes). Twelve percent of patients with MULTI had concomitant diabetes (88 % had no concomitant diabetes). Statistical analysis showed that the occurrence of diabetes was significantly higher in POLY than in MULTI ($p = 0.0006$).

Conclusions: We believe that our study provides reliable data on QoL in patients with neuropathies and that it may represent a step forward to understand and quantify the extent of patients' disability and deterioration of their life due to POLY and MULTI. Further studies are necessary to provide useful data for clinical practice and they should be focused on the assessment of QoL in the several kinds of neuropathies, on the evaluation of the natural history of the different kinds of neuropathies and on the evaluation of the effects of therapies.

O166

Diagnostic usefulness of anti-glycolipid IgM antibodies in chronic dysimmune neuropathies

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IgM antibodies against several neural antigens have been associated with chronic dysimmune neuropathies (PN) including anti-myelin-associated glycoprotein (MAG) IgM in predominantly sensory demyelinating PN associated with IgM monoclonal gammopathy (PN+IgM), anti-GM1, -GM2 or -GD1a IgM in multifocal motor neuropathy (MMN) or other motor neuropathies (MN) and anti-GQ1b and -GD1b IgM in sensory PN+IgM and anti-sulfatide IgM in predominantly sensory axonal PN or demyelinating PN+IgM. While the clinical correlate and usefulness of anti-MAG IgM antibodies are well established, these are less definite for anti-glycolipid IgM antibodies. To determine the possible diagnostic value of anti-glycolipid antibodies in dysimmune neuropathies we reviewed their clinical correlate in 443 consecutive MAG-negative patients with neuropathy or related clinical syndromes examined in our Neuropathy Clinic and tested for these antibodies in our laboratory since 1985. 205 patients (46 %) had a definite or possible chronic dysimmune PN while 238 (54 %) had a non-dysimmune (73) or undiagnosed (82) PN or closely related diseases (84). High anti-GM1 IgM (> 1/320 by ELISA) were detected in 31 patients, 20 of whom (65 %) had a dysimmune PN that in 11 was MMN or MN (35 %), while 11 had other PN or related diseases including 4 (13 %) with MND. High (> 1/320) anti-GM2 (12 patients), -GD1a (6 patients), and -GD1b or GQ1b (6 patients) IgM were associated in all but two patients with a PN of unknown etiology, with a dysimmune PN (92 %). Of the 5 patients with high anti-sulfatide IgM (> 1/8,000 by ELISA), 4 (80 %) had a dysimmune PN and one a PN of unknown etiology. IgM antibodies to one or more glycolipd were significantly more frequent in patients with dysimmune PN (22 %) than with other PN or related diseases (6 %)($p < 0.0001$). Their presence had a specificity and positive predictive value for a dysimmune PN of 94 % and 77 % respectively. These data confirm that anti-glycolipid IgM antibodies may help identifying patients with a chronic dysimmune neuropathy even if they are not strictly associated with a definite clinical syndrome.

O167

Clinical and electrophysiological data of chronic ataxic neuropathy associated with anti-disialosyls antibodies

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We retrospectively studied 15 patients in whom we found anti GD1b or GQ1b antibodies. 7 men and 8 women with a mean age of 52 years were analysed. 14 patients had a chronic ataxic neuropathy, one suffered from repetitive episodes of ophthalmoplegia and bulbar weakness.

Ataxia was always associated with areflexia except in one case. Hypopallesthesia was constant. Weakness was present in 5 patients and 5 had cranial nerves involvement. Clinical course was subacute in 4 patients, relapsing in six and chronic progressive in the others.

Anti-GQ1b antibodies were detected in 5 patients, anti GD1b in 10, never in association. An IgM MGUS was present 7/15 and cold agglutinins 3/8. Fully biological CANOMAD criteria as proposed by Willison et al. were fulfilled in only 3 patients.

CSF examination demonstrated an elevated protein level in 6 patients.

Electrophysiogical study showed axonal sensitive neuropathy or neuronopathy in 11 patients, normal NCV suggesting pre-ganglionic lesions in 3 patients and isolated F-waves abnormalities in the others. A typical pattern of demyelinating neuropathy never occurred. Sural nerve biopsy performed in 4 patients revealed axonopathy in 2 patients, a mixed pattern of axonal and demyelinating lesions in one and was normal in one.

IVIg was administrated to 8 patients at least for 2 courses and led to a marked improvement in half of the cases.

In conclusion, the most typical clinical pattern associated with anti GD1b or GQ1b antibodies is a chronic or subacute ataxic polyneuropathy without any other neurological signs. The neurophysiological pattern is variable: pre-ganglionic radicular pattern with normal NCV, pure sensory

neuronopathy or axonal motor and sensory polyneuropathy. Isolated relapsing oculomotor and/or bulbar involvement mimicking myasthenia may also occur but is much less frequent. A full CANOMAD syndrome is in fact rare and even IgM MGUS is present only in half of the cases. IgIV may be a very effective therapy suggesting that screening for these antibodies is required when exploring an ataxic syndrome even in case of normal NCV.

O168
Charcot-Marie-Tooth: Italian multicentre study on quality of life and disability. Influence of genotype and phenotype
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Over the last two decades, clinical and public health researchers have emphasized the need for a thorough evaluation of concepts such as Health Related Quality of Life (QoL) to study the impact of chronic illnesses and their treatments on the patient's life.

Charcot-Marie-Tooth (CMT) patients develop progressive weakness and sensory disturbances, becoming sometimes severely disabled even at very young age. In CMT clinic, neurophysiologic, pathologic and genetic evaluation are considered fundamental to assess nerve involvement and diagnosis, but how these findings are related to HRQoL and disability is not assessed. Although the clinical features suggest that CMT patients have deterioration of Quality of Life, no wide multicentre studies were performed to evaluate it.

We performed a prospective multicentre study on patients with CMT by using multiple measures of CMT. The aim of the study was to assess QoL and disability of patients with CMT, in a large and well-represented sample, and to evaluate the relationships between diagnostic and severity conventional parameters (clinical, neurophysiological, neuropathologic and genetic measures) and the patients' perception of their own quality of life and disability.

We consecutively enrolled 212 patients: 84 male, 128 female, mean age 42.5 yrs, SD 15.0, range 8–90. Distribution of age was normal. Mean age of onset of symptoms in the whole patient sample was 22.7 yrs (SD 16.7, range 1–69). In 86 % of the cases it was possible to identify familiarity.

With regard to disability, about 8.2 % of patients were not able to walk independently and only 40.1 % of patients were able to walk independently and normally. With regard to the QoL pattern of the enrolled sample, all of the SF-36 QoL domains were highly significantly deteriorated ($p < 0.001$) in the enrolled patient sample with respect to the Italian normative sample. Statistical multiple regression analysis identified the variables that cause deterioration of QoL and disability.

O169
Membrane function of regenerated axons in mouse
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We have recently shown "in vivo", by means of threshold tracking (a non-invasive electrophysiological technique) in cat that the membrane excitability of regenerated axons remains persistently abnormal and provided indirect evidence that regenerated axons are hyperpolarized, most likely due to hyperactivity of the Na+/K+ pump (Moldovan & Krarup, J. Physiol. 2004560.3:807–19). To further test this hypothesis, the aim of the present study was to develop a comparable model in mouse to combine electrophysiological methods with pharmacological and genetic manipulations. Sciatic nerves of 8 mice (18–20 g) were crushed unilaterally ~1 cm above the knee. The contralateral unlesioned nerves served as controls. Multiple excitability tests were carried out monthly under anesthesia by stimulation at ankle and recording of the CMAPs from plantar muscles. One month after crush the regenerated CMAPs were biphasic ~2 mV (25 % of control) and accessible for threshold tracking. Regenerated nerves showed abnormal passive membrane properties: prolonged latency, increased chronaxie and decreased input conductance. Additionally, the supernormal period of excitability recovery (indicating internodal discharge during nodal repolarization) was completely abolished. The threshold changes during electrotonus were more pronounced than in controls, notably the threshold increase during hyperpolarization. Two months after crush, the response to depolarizing electrotonus became undistinguishable from controls while the response to hyperpolarizing electrotonus recovered only partially. This discrepancy indicated that active membrane properties were also abnormal in regenerated mouse nerves consistent with resting membrane hyperpolarization as indicated by the presence of inward rectification. These abnormalities were largely similar to those observed in cats. The greater recovery in hyperpolarizing threshold electrotonus observed in mice may however reflect differences in Na+/K+ pump contribution to membrane potential between species.

O170
Burning mouth syndrome: a new small-fibre sensory neuropathy
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Burning mouth syndrome (BMS) is a chronic disorder affecting about 1 million people in the United States, mainly women in the 5th–7th decade. It is characterized by persisting painful symptoms involving the tongue, mainly tip and anterior two-thirds, hard palate, lips, and alveolar ridges. Systemic and local diseases were claimed to play a causal role, though most often BMS was attributed to psychological causes. Primary neuropathic dysfunction was recently suggested. However, the primary site of pathology was not identified and no diagnostic test was available so far. We aimed at assessing if BMS is associated with changes in the innervation of the tongue. We examined 12 patients with unremitting sensory disturbances in the mouth for at least 6 months. Patients with oral infections, contact sensitivity, systemic disorders or peripheral neuropathies were excluded. All patients and 9 healthy subjects underwent a 3-mm punch biopsy at the anterolateral aspect of the tongue close to the tip. Sections were immunostained with anti-protein-gene-product 9.5 (PGP 9.5) antibodies. Two blinded observers counted the number of epithelial nerve fibers (ENF) arising from sub-papillary bundles and obtained the linear innervation density (ENF/mm). Densities were compared between groups by analysis of variance (ANOVA). Further sections were immunoassayed with anti-unique β-tubulin (TuJ1) antibodies, anti-myelin basic protein (MBP), and anti-peripheral myelin protein 22 (PMP22) antibodies for cytoskeletal, myelin sheath, and Schwann cell labeling. Confocal microscope studies between PGP 9.5-MBP and TuJ1-PMP22 were performed. BMS patients showed a lower ($p = 0.0004$) ENF density (10.29/mm; 95 % CI 15.89–5.01) than controls (27.73/mm; 95 % CI 37.71–20.35). There was a trend toward correlation between loss of ENF and duration of symptoms. Co-localization studies revealed that ENF are naked axons with no Schwann cell ensheathment. Our findings unambiguously demonstrated that BMS patients have a small fiber sensory neuropathy of the tongue and that biopsy can be used to assess the diagnosis.

Session 27

Neuro-oncology

O171
Intrathecal polyspecific immune reaction against neurotrophic viruses discriminates between multiple sclerosis and paraneoplastic neurological syndromes
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Background: The detection of locally synthesized CSF Ig is helpful in the diagnosis of inflammatory CNS diseases. Different from oligoclonal IgG bands (OCB), which can be present in infectious as well as in non-infectious diseases, the detection of a combined intrathecal humoral immune response against neurotropic viruses like measles (M), rubella (R), and/or VZV (Z) (MRZ reaction, MRZR) is thought to be restricted to autoimmune (AI)-mediated diseases of the CNS. MRZR has been shown to be highly sensitive for MS (up to 89 %). Its specificity for this disease, however, remains uncertain. Paraneoplastic neurological syndromes (PNS) are rare AI disorders of the CNS in px with tumors. PNS often follows a subacute course, but may also be slowly progressive or even spontaneously relapsing-remitting, thereby mimicking MS. In PNS OCBs can be frequently detected in CSF. The presence of an intrathecal antiviral immune response as seen in MS, however, has not been assessed in PNS so far.

Objectives: To assess the frequency of polyspecific ab against M, R and Z in the CSF of px w/PNS (n = 35).

Methods: Ab levels were quantified by ELISA.Albumin and IgG conc were determined nephelometrically. The intrathecal synthesis of ab to M, R and Z was detected by calculation of the ab index (AI) as AI = Qspec/QIgG. In the case of QIgG > Qlim the corrected AI was calculated as AI = Qspec/Qlim. An AI > 1.5 was considered to indicate a significant intrathecal IgG production to the pathogen studied.

Results: 35 px w/various PNS were included in our study (m: f = 1:2; 4–84a, median 69a). Anti-neuronal serum ab found in these px included anti-Hu (n = 20), anti-Yo (n = 4), anti-Ma (n = 6), anti-Ta (n = 3), anti-Ri (n = 1) and anti-Tr (n = 1). Intrathecal IgG production was detectable in 49 %. However, in none of our px a positive MRZ reaction as defined by a combination of at least two positive AIs was found. A slight (AI > 1.5) monospecific immune reaction was present in 6 %, a reliable monospecific AI increase (AI > 2.0) in 15 %. In contrast, 15/17 (88 %) consecutive MS px were tested positive for MRZ (trispecific response in 73 %, bispecific in 27 %). Median AI in PNS was 0.88 for M, 1.01 for R, and 1.13 for Z.

Conclusions: MRZR is reported to be detectable in up to 89 % of MS px, but was not detectable in any PNS px assessed in our study. Our results enhance the discrimination of MS and PNS by CSF analysis. It has to be stressed, however,that PNS should never be excluded on the sole basis of CSF findings and larger studies are needed to confirm our results.

O172

Efficacy of radiation therapy on seizures in low-grade astrocytomas

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The role of radiation therapy in low grade astrocytomas is still controversial, and seizure control represents the most relevant clinical problem. There are some reports in the literature suggesting that radiation therapy might be effective in reducing seizure frequency in patients with inoperable low grade gliomas. The objective of this study was to analyse in a retrospective series of patients with supratentorial histologically verified grade II astrocytomas and persisting seizures despite conventional AEDs, the response of epilepsy to conventional radiotherapy. The study population (1985–2001) included 25 patients, ages 20 to 54 years (median 35), 16 men and 9 women. Previous surgery consisted of a biopsy in 9 patients, a partial/subtotal resection in 13 patients and a total resection in 3 patients. The seizure frequency varied from > 1 per day to > 1 per month, and the type was as follows: partial simple in 9, partial complex in 9 and generalized in 7. Twenty-two tumors were non-enhancing on CT or MRI, whereas 3 only were enhancing. Patients received external radiotherapy either adjuvantly or at tumor progression in conventional fractionation with total doses ranging from 40 to 60 Gy (median 54). Overall, 19 patients (76 %) had a significant reduction (> 50 % decrease) in seizure frequency, with 2 patients seizure free, whereas 5 patients (20 %) had no significant change and 1 (4 %) had an increase in seizures. Basing on Macdonald's criteria of response, we observed 6 PR (24 %), 17 SD (68 %) and 2 PD (8 %). When correlating the clinical with the radiological response, among patients who had a significant reduction of seizure frequency (19), 4 (21 %) had a PR, 13 (68 %) a SD and 2 (11 %) a PD, whereas among patients who had no significant change of seizure frequency (5), 2 (40 %) had a PR and 3 (60 %) a SD. In conclusion, this study confirms in a larger series of patients that conventional radiotherapy is able to significantly reduce the seizure frequency in a high proportion of patients with grade II astrocytomas. Moreover there does not seem to exist a correlation between the seizure reduction and tumor response on CT or MRI.

O173

Acute confusion in cancer patients

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Background and Objective: Acute alteration of cognitive functions without significant change of arousal, also named delirium, is common in systemic diseases. The aim of this study was to assess the prevalence of this condition in patients admitted to a department of general medical oncology.

Patients: One hundred consecutive cancer patients who fulfilled DMS-IV criteria of delirium were investigated from September 2002 to September 2003. They represented 11.8 % of the admissions. Ninety-one patients suffered their first episode of confusion. Over three quarter of the patients had progressive mostly metastatic disease.

Results: Thirty-six patients had structural brain lesion. Thirty-two were metastatic (18 parenchymal, 6 leptomeningeal and 8 both parenchymal and leptomeningeal), three had recent intra-cranial haemorrhage and one patient had limbic encephalitis. These structural lesions were considered as the main cause of confusion in all the 36 patients. In 64 cases, no structural lesion was found. In 6 of the 64 patients, the cause of confusion remained undetermined, and one patient suffered acute psychosis. In 57 patients, confusion was due to a metabolic or toxic encephalopathy or sepsis, and most patients had more than one potential cause of encephalopathy. Confusion was reversible in about one third of the patients, predominantly in those without structural brain disease.

Conclusion: In cancer patients metabolic and toxic encephalopathy and sepsis cause acute confusion more often than structural lesions. Most patients had more than one potential cause of confusion. In patients with structural brain lesion, confusion was usually irreversible and had a poor survival prognosis.

O174

Epileptic seizures during follow-up of patients treated for primary brain glioma

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Objective: Epileptic seizures are common in patients with primary brain tumors and may significantly alter the quality of life of these patients. The aim of our study is to determine the presentation, the incidence and the severity of epileptic seizures in follow-up of patients treated for primary brain tumors.

Patients and Results: Two hundred thirty-four consecutive patients attending an outpatient clinic for chemotherapy of a supra-tentorial brain tumor were examined. Hundred eighty-three (78.2 %) experienced tumor-related seizures, and 51 (22.8 %) did not. All epileptic patients were on anti-epileptic drugs (AEDs). Compared with patients without epilepsy, the group of epileptic patients was characterized by a higher proportion of low-grade gliomas ($p < 0.001$) and of cortical tumor location ($p < 0.001$). In 158 (86.4 %) of the 183 epileptic patients seizures were an early manifestation of the disease, and only 25 (13.6 %) individuals acquired epilepsy in the course of the malignant disease. Generalization occurred in 50 % of early seizures, but in only 19.1 % of patients with seizures persisting after the initiation of AEDs and specific anti-tumor therapies. The reduction of seizure generalization was statistically significant ($p = 0.001$). Despite AED-administration and various anti-tumoral treatments, half of the patients had seizure within one month and two-thirds within three months preceding the last evaluation.

Conclusion: Most tumor-related seizures first appear in early course of the disease, usually as a presenting manifestation. AEDs combined with specific anti-tumoral treatments significantly reduce the rate of seizure generalization. However, the majority of patients continue to present mostly focal epilepsy during therapeutic follow up.

O175

Temozolomide as initial treatment for progressive low grade oligodendrogliomas: treatment results and correlation between genetic profile and MGMT protein expression

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Loss of heterozygosity (LOH) on chromosomes 1p and 19q has been associated with favorable response to chemotherapy and good prognosis in oligodendrogliomas. The DNA repair enzyme O6-methylguanine DNA methyltransferase (MGMT) may induce resistance to DNA-alkylating drugs. Recent studies showed that temozolomide (TMZ), an oral alkylating agent, has efficacy in progressive low-grade oligodendrogliomas (LGO). Yet, limited data are available regarding the association between 1p/19q profile and MGMT protein expression in these tumors.

Objective: To evaluate the response of progressive LGO to TMZ and to assess the association between 1p/19q profile and MGMT protein expression.

Methods: Adult patients, whose MRI findings and/or clinical deterioration were compatible with progressive LGO, were eligible for the study if they were radiotherapy naïve. TMZ dose was 200 mg/m^2/d for 5 days repeated every 28 days. Clinical and MRI data served for evaluation of outcome and Kaplan-Meier estimates were used to assess median time to tumor progression (TTP) and progression-free survival (PFS). 1p/19q status was evaluated from paired tumor-blood DNA samples using PCR-based microsatellite analysis. MGMT protein expression was studied in paraffin embedded tumor sections by immunohistochemistry.

Results: 28 patients (median age: 38 range 17–77) were enrolled. Median time between tumor diagnosis and TMZ treatment was 33.5 months

(range: 1–133). Median number of TMZ cycles/patient was 12 (range: 2–24). Marked clinical improvement was recorded in 15 patients (54 %), an objective response in 17(61 %) with 7 minor responses, stable disease in 10 (36 %) and progressive disease in one patient. Median TTP is 31 months with PFS of 70 % at 24 months. LOH of 1p and/or 19q was found in 14/16 tumors of whom 11 improved on TMZ and 3 had stable disease. Of the 2 tumors with no LOH one had stable disease and the other progressed. MGMT protein expression was evaluated in 16 LGO whose LOH status showed 13 co-deletions at 1p/19q and 3 had an intact 1p chromosome. LGO with an intact 1p demonstrated high expression of MGMT (> 50 % nuclear staining) as opposed to tumors with 1p loss that exhibited a relatively low MGMT (0–50 % nuclear staining). MGMT expression was significantly associated with 1p loss ($p < 0.0004$).

Conclusions: TMZ is active in progressive LGO. 1p/19q deletions are associated with response to TMZ and MGMT protein expression significantly correlates with the allelic loss on 1p chromosome.

O176

Anti-tumour vaccination of patients with GBM: a pilot study

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Purpose: Prognosis of patients with glioblastoma is poor. Therefore, we analyzed in glioblastoma patients whether anti-tumor vaccination with a virus-modified autologous tumor cell vaccine is feasible and safe. Also, we determined the influence on progression-free (PFS) and overall survival (OS) and on vaccination-induced anti-tumor reactivity.

Patients and Methods: In a non-randomized study, 23 patients were vaccinated and compared to non-vaccinated controls (n = 87). Vaccine was prepared from patient's tumor cell cultures by infection of the cells with Newcastle Disease Virus (NDV), followed by ƒx-irradiation and applied up to 8 times. Anti-tumor immune reactivity was determined in skin, blood and relapsed tumor by DTH skin reaction, ELISPOT-assay and immunohistochemistry, respectively.

Results: Establishment of tumor cell cultures was successful in about 90 %. After vaccination, we observed no severe side effects. Median PFS of vaccinated patients was 40 weeks (versus 26 weeks in controls, log-rank test, $p = 0.024$), median OS of vaccinated patients was 100 weeks (versus 49 weeks, log-rank test, $p < 0.001$). 45 % of the controls survived one year, 11 % two years, and there were no long-term survivors ("d3 years). In the vaccinated group, 91 % survived one year, 39 % two years, and there were 4 % long-term survivors. In the vaccinated group, immune monitoring revealed significant increases of DTH reactivity, numbers of tumor-reactive memory T-cells and of CD8+ tumor-infiltrating T-lymphocytes in secondary tumors.

Conclusion: Postoperative vaccination with virus-modified autologous tumor cells appears to be feasible, safe, and seems to improve the prognosis of patients with glioblastomas. This could be substantiated by the observed anti-tumor immune response.

POSTER SESSIONS

Poster session 1

Child neurology

P177

Intracranial haemorrhages of premature newborn

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Intracranial hemorrhages of newborn and children of early age include a large group of pathological states, heterogenic etiology and clinical condition. The main cause of intracranial hemorrhage of mature newborn is labor trauma, and for the immature newborn chronic and acute hypoxia, or sometimes traumatic changes. Skull trauma is the main cause of hemorrhages between the age of 1 and 3.

The purpose of the investigation was to determine the treatment and diagnostic approach to the intracranial hemorrhages of premature newborns.

835 immature newborns were examined with prenatal hypoxia and asphyxia and were treated at the Republic Crimean Children Clinical Hospital during 2001 to 2004. The massive intracranial hemorrhages were verified. The particular feature of examined patients was the combination of various kinds of hemorrhages. Most of the attention was paid which excluding the traumatic squeezing of brain by intracranial hemorrhage, are the sanation of liquor, prevention and treatment of this severe complication such as post-hemorrhage hyprocephalia. 34 patients had been operated by using ventricular-peritoneal shunting.

The particular feature of the pathogenesis of intracranial hemorrhages which it is necessary to investigate the ways to compensate the cerebral circulatory damage in the condition of ischemia and hypoxia, which are caused by complex pathological factors. In spite of the poly-etiological development of the intracranial hemorrhages in immature newborns, one of the main causes is the insufficiency of the anesthomosis between the short and long branches of basic cerebral arteries, basilar and periventricular vessels, insufficiency of the germinal matrix of the vessels. The perspective investigation is the improvement of cerebral circulation in extracranial segments of the cerebral vessels and collateral circulation.

P178

Bone marrow stromal cells as a tool for cell therapy for lysosomal storage disorders with neurologic involvement

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Bone marrow stromal cells (BMSCs) are multipotent stem cells, like those residing in the bone marrow. They are capable of differentiation into osteoblasts, chondroblasts, and adipocytes in the respectively appropriate environment in which growth signals are rendered. Particularly, BMSCs are also considered progenitors of non-hemopoietic cells (myoblasts, neural cells). Therefore, BMSCs have been proposed as a therapeutic tool for genetic disease. We are evaluating the potential of BMSCs to serve as effective vehicles for both cell and gene therapy for Tay-Sachs disease, a genetic lysosomal storage disorder with neurological involvement.

Therapy for neurodegenerative lysosomal storage Tay-Sachs disease requires an active Hexosaminidase A (Hex A, E. C. 3.2.1.52) production in the central nervous system and a therapeutic approach whose effect would prevent/revert the progression of the neurodegeneration. To this end, we aim to combine the efficacy of the gene therapy strategy by using a viral vector encoding for Hex A alpha-subunit to restore Hex A activity and the therapeutic potential of BMSCs to repair the neurodegenerated central nervous system.

We produced murine BMSCs by flushing the femurs of adult Tay-Sachs and wild-type mice. mBMSCs in culture start as a fibroblast-like colony, and then grow as a monolayer. In this experimental condition, we selected first a heterogeneous cell population then we selected by limiting dilution. We transduced BMSCs from TS mice with the retroviral vector encoding for the alpha-subunit of Hexosaminidase A (L-alpha-HexTN) to produce Hexosaminidase A and to restore the metabolic defect.

We demonstrated the ability of these cells to give rise in vitro to neural cells by treating selected BMSCs clones with appropriate medium culture containing FGF or PDGF as specific mitogens.

P179

Clinical, biochemical and genetic investigation in children with mitochondrial dysfunction

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Mitochondrial diseases (MD) represent a heterogeneous group of multisystem disorders which preferentially affect tissues with high energetic demands such as muscle, nervous system, heart, endocrine system, etc. and are often manifested in children. Because the brain is highly dependent on oxidative metabolism, encephalopathy and epilepsy are common manifestations of many of these conditions. They are caused either by a mutation in the maternally inherited mitochondrial genome, or by nuclear DNA-mutation. These disorders may present with a huge variety of symptoms, even if the same mutation is involved. In our investigation we examined 24 children (36 months–16 years) suspected of having MD. Elevated serum lactate level, clinical features such as hypotonia, exercise intolerance, hyperactivity, cardiac dysrhythmia were observed in every patient. Migraine headaches, vomiting, delayed speech also affected most of these patients. Breath-holding spells and/or febrile convulsions were seen in 3 of the pa-