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Peer Tfelt-Hansen

The effect of ergotamine on the arterial system in man

A doctoral thesis in medical science based on the following works:
Tfelt-Hansen P, Eickhoff J H and Olesen J. The effect of single dose ergotamine tartrate on peripheral arteries in migraine patients: Methodological aspects and time effect curve. *Acta Pharmacol Toxicol (Copenh)* 1980; 47: 151–156.
Tfelt-Hansen P and Olesen J. Arterial response to ergotamine tartrate in abusing and non-abusing migraine patients. *Acta Pharmacol Toxicol (Copenh)* 1981; 48: 69–72.
Tfelt-Hansen P, Eickhoff JH and Olesen J. Mechanism of ergotamine-induced decrease of peripheral systolic blood pressure in man. *Acta Pharmacol Toxicol (Copenh)* 1982; 51: 122–125.
Tfelt-Hansen P, Østergaard JR, Gøthgen I, Jacobsen E, Rasmussen JP and Husum B. Nitroglycerin for ergotism. Experimental studies in *vitro* and in migraine patients and treatment of an evert case. *Eur. J. Clin. Pharmacol.* 1982; 22: 105–109.
Tfelt-Hansen P, Kanstrup I-L, Christensen NJ and Winkler K. General and regional haemodynamic effects of intravenous ergotamine in man. *Clin.* Sci. 1983; 65: 599–604.
Tfelt-Hansen P and Manniche PM. Dose-response curve for the ergotamine-induced decrease of peripheral systolic blood pressure in man. *Acta Pharmacol Toxicol (Copenh)* 1984; 55: 238–241.
Tfelt-Hansen O and Paa1zow L. Intramuscular ergotamine: plasma levels and dynamic activity. *Clin.Pharmacol. Ther.* 1985; 37: 29–35.

The constrictor effect on human leg arteries after i.v. or i.m. ergotamine tartrate was measured in 6 studies as decreases in peripheral systolic BP (measured with strain-gauge plethysmography). Previously, the vascular effect of ergotamine was, based on regional blood flow in animals, described as short-lived, a seemingly "comforting fact" for its use in human therapy. In man the effect on arteries developed slowly over hours and was still present the next day. In contrast, the increase of total peripheral resistance, an effect on arterioles, was only present for a few hours after ergotamine.

The sustained constrictor effect on arteries of ergotamine was used as a model for the rare ergotism and it was shown that NTG-infusion is effective in counteracting the effect of ergotamine.

Finally, the correlation between arterial effect and pharmacokinetics of i.m. ergotamine 0.5 mg was evaluated in the effect-compartment model of L.B Sheiner et al. There was a huge discrepancy in the time-effect curves for plasma concentrations of the drug and for its effect on arteries. Thus, T_{max} for plasma concentrations of ergotamine was 30 min; whereas the T_{max} for arterial effect was 6 h. Ergotamine could not be detected in plasma after 30 h, at a time when the constrictor effect on arteries was still pronounce. A clinically interesting observation was the 30-fold variability among the 10 migraine patients in the sensitivity of leg arteries to ergotamine.

Kai Jensen

Extracranial blood flow, pain and tenderness in migraine. Clinical and experimental studies

A doctoral thesis in medical science based on the following works:
Jensen K, Olesen J. Temporal muscle blood flow in common migraine. *Acta Neurol Scand* 1985; 72: 561–570.
Jensen K, Bülow P, Hansen H. Experimental tooth clenching in common migraine. *Cephalalgia* 1985; 5: 245–251.
Jensen K, Andersen H Ø, Olesen J, Lindblom U. Pressure pain threshold in human temporal region. Evaluation of a new pressure algometer. *Pain* 1986; 25: 313–323.
Jensen K. Subcutaneous blood flow in the temporal region of migraine patients. *Acta Neurol Scand* 1987; 75: 310–318.
Jensen K, Tuxen C, Olesen J. Pericranial muscle tenderness and pressure pain threshold in the temporal region during common migraine. *Pain* 1988; 35: 65–70.
Jensen K, Tuxen C, Pedersen-Bjergaard U, Jansen I, Edvinsson L, Olesen J. Pain and tenderness in human temporal muscle induced by bradykinin and 5-hydroxytryptamine. *Peptides* 1990; 11:1127–1132.
Jensen K, Tuxen C, Pedersen-Bjergaard U, Jansen I. Pain, tenderness, wheal and flare induced by Substance-P, bradykinin and 5- hydroxytryptamine in humans. *Cephalalgia* 1991; 11: 175–182.

Jensen K, Norup M. Experimental pain in human temporal muscle induced by hypertonic saline, potassium and acidity. *Cephalalgia* 1992; 12: 101–106.

Migraine pain has traditionally been ascribed to dilatation of primarily extracranial arteries. Such dilatation has, however, not been demonstrated so far. Studies of microcirculation reveal no major hyperperfusion or ischemia in the temporal muscle or the subcutaneous tissue in the temporal region during attacks of migraine. However, a reduction in the orthostatic reactivity of the subcutaneous arterioles was observed on the side of the headache. Increased tenderness of the pericranial myofascial tissues is observed during migraine attacks, particularly on the side of the headache. Increased tension of pericranial muscles on the other hand is not a constant finding and migraine attacks are not induced by experimentally increased tension of the temporal and masseter muscles. Extracranial pain and tenderness may, however, be induced experimentally by intramuscular injections of hypertonic saline and potassium chloride as well as of endogenous substances like bradykinin with 5-hydroxytryptamine and bradykinin with substance P. The extracranial arteries and myofascial structures are both supplied by unmyelinated trigeminal sensory nerve fibers containing a variety of neuropeptides which are released during migraine attacks. Axonal reflexes between extracranial arteries and neighboring myofascial tissues as well as referred pain mechanisms may account for the observed tenderness during migraine attacks.

Birthe Krogh Rasmussen

Epidemiology of headache

A doctoral thesis in medical science based on the following works:

Rasmussen BK, Jensen R, Schroll M, Olesen J. Epidemiology of headache in a general population – a prevalence study. *J Clin Epidemiol* 1991; 44: 47–57.
Rasmussen BK, Jensen R, Schroll M, Olesen J. Interrelations between migraine and tension-type headache in the general population. *Arch Neurol* 1992; 49: 914–918.
Rasmussen BK, Jensen R, Olesen J. A population-based analysis of the diagnostic criteria of the International Headache Society. *Cephalalgia* 1991; Il: 129–134.
Rasmussen BK, Jensen R, Olesen J. Questionnaire versus clinical interview in the diagnosis of headache. *Headache* 1991; 31: 290–305.
Rasmussen BK, Jensen R, Olesen J. Impact of headache on sickness absence and uilisation of medical services: a Danish population study. *J Epidemiol Community Health* 1992; 46: 443–446.
Rasmussen BK, Olesen J. Symptomatic and non-symptomatic headaches in a general population. *Neurology* 1992; 42: 1225–1231.
Rasmussen BK. Migraine and tension-type headache in a general population. Psychosocial factors. Int *J Epidemiol* 1992; 21: 1138–1143.
Rasmussen BK. Migraine and tension-type headache in a general population: Precipitating factors, female hormones, sleep pattern and relation to lifestyle. *Pain* 1993; 53: 65–72.
Rasmussen BK, Olesen J. Migraine with aura and migraine without aura: an epidemiological study. *Cephalalgia* 1992; I 2: 221–228.

This study represents the first prevalence study of specific headache entities in a representative general population based on a structured interview and examination by a physician and using internationally accepted operational diagnostic criteria. The headache classification of the International Headache Society (IHS) has given operational diagnostic criteria for all headache disorders and has thus provided new opportunities for valid epidemiologic al headache research. One thousand 25–64 year-old men and women, who lived in the western part of Copenhagen County were randomly drawn from the Danish National Central Person Registry. All subject s were invited to a general health examination focusing on headache. The participation rate was 76% and information about non-participants was obtained by telephone interview s or mailed questionnaires. In only 5% of the study population information lacked. Non-participants did not differ from participants to any appreciable degree.

The aims of the study were to assess the prevalence's of the different forms of headache using the IHS-criteria and to provide descriptive data concerning symptomatology, precipitating factors, impact of female hormones, use of medical services and work consequences of the head ache disorders and to describe various factors associated with the disorders.

The lifetime prevalence of migraine was 16%, and of tension-type headache 78%.

Prevalence of migraine in the previous year was l0% and of tension-type headache 74%. Differences according to sex were significant with a male: female ratio of l:3 in migraine, and 4:5 in tension-type headache. It is shown that an influence of female hormones may be responsible for this female preponderance. The prevalence of tension-type headache decreased with increasing age, whereas migraine showed no corre1ation to age within the studied age interval. The age at onset of migraine was mainly in the second and third decennia, the age at onset of tension-type headache mainly in the second decennium.

Among those with migraine in the previous year 25% had it 8–14 days a year and 16% had it more than 14 days a year. Of the tension -type headache group 24% had it 8–14

days a year, and 41% more than 14 days a year. The prevalence of chronic tension type headache was 3% (i.e. headache 2180 days a year).

The most common precipitating factor of both migraine and tension-type headache was stress and mental tension. Also alcohol, smoking and weather changes were frequent precipitants, the two last mentioned especially in subject s with tension-type headache.

In migraine's, pain was of a pulsating quality in 78%, severe in 85%, unilateral in 62%, and aggravated by routine physica1 activity in 96%. Tension -type headache was of a pressing quality in 78%, mild or moderate in 99%, bilateral in 90%, and 72% had no aggravation by physical activity. The accompanying symptoms of nausea, photo and phonophobia occurred frequently and were usually moderate or severe in migraine subjects, and if present in subject s with tension-type headache, they were usually mild. The IHS-classification is evaluated and suggestions for possib1e modifications of the criteria are given.

The study supports that migraine and tension-type headache are separate clinical entities although they may coexist and interrelate. Positive support for the existence of two separate diseases are found in specific symptom clustering in both migraine and tension-type headache, in a different relationship between frequency and severity in migraine respectively tension -type headache, in a different sex distribution, in a different distribution of age at onset and finally in different endogenous and exogenous risk indicators. It is stressed that longitudinal follow-up studies are necessary to demonstrate how the headache disorders progress, improve, or interrelate over time. No support is given to introduce new categories of headache such as 'combination headache', 'mixed headache' or 'interval headache '.

The study supports that migraine without aura (MO) and migraine with aura (MA) are different subforms of the same disorder. Lifetime prevalence of MO was 9%, M:F ratio 1:7. Lifetime prevalence of MA was 6%, male:female ratio 1:2. Females, but not males, were significantly more likely to have MO than MA. MO was more strongly related to female hormones than MA. Visual disturbances were the most common aura phenomenon occurring in 90% of subjects with MA. The pain phase of MO and MA was found to be very similar while differing initiating mechanism s and initial phases may occur.

Only half of migraineurs and one-sixth of subjects with tension- type headache consulted their general practitioner because of headache and even less consult a specialist. In both migraineurs and in subjects with tension-type headache the consultation of a general practitioner was positively correlated to the frequency of attacks. The consultation rate was higher among women than among men. These consultation rates reflect the selection of cases in clinic populations and emphasize that assessment of risk factors for the headache disorders should not only be performed in studies comprising clinically diagnosed cases.

The consultation rates of chiropractors and physiotherapists were 5–8%. Hospitalization and supplementary laboratory investigations due to headache were rare (<3%). Work absence because of migraine occurred in 43% of employed migraineurs and in 12% of employed subjects with tension-type headache in a year. Most common was 1–7 days off work in a year because of the headache. The total loss of workdays per year due to migraine in the general population was estimated to 270 days per 1,000 persons. For tension-type headache the corresponding figure was 820.

There is no clear evidence of any association between sociodemographic variables and migraine or tension-type headache. Tension-type headache was related to a series of psychosocial variables and to level of physical activity while migraine was not. The results suggest that migraine is primarily a constitution al disorder and tension-type headache a more complex phenomenon influenced by several psychosocial factors.

The limitations of cross-sectional data to point out risk factors with sufficient certainty are stressed. Longitudinal follow-up studies are the most important challenge in future epidemiological headache research.

Michael Bjørn Russel

A clinical investigation of migraine

A PhD thesis based on the following works:
Russell MB, Rasmussen BK, Brennum J, Iversen HK, Jensen R, Olesen J. Presentation of a new instrument: The diagnostic headache diary. *Cephalalgia* 1992; 12: 369–374.
Russell MB, Iversen HK, Olesen J. Improved description of the migraine aura by a diagnostic aura diary. *Cephalalgia* 1994; 14: 107–117.

The purpose of the present PhD thesis was to investigate the clinical characteristics of migraine without aura (MO) and migraine with aura (MA). The operational diagnostic criteria of the International Headache Society were used. Since migraine is a subjective complaint, a clinical investigation relies exclusively on the information given by the study population. The most precise description can be ascertained by prospective records. Such records are complicated by a low compliance. Representative retrospective data can be ascertain from the general population, but this information is influenced by the threshold of recall probably varies with the severity and recency of the condition. Thus, a combination of prospective and retrospective data gives the most complete clinical description of migraine.

Prospective records. A diagnostic headache diary and a diagnostic aura diary were applied on 61 and 20 migraineurs from a research headache clinic. The diaries are for

prospective records of headaches and aura symptoms. The diagnostic headache diary had records of more diverse types of headache than the clinical interview, but all migraineurs had recorded at least one migraine attack, indicating good accordance between the clinical interview and the headache diary. The diagnostic aura diary records indicate that aura symptoms precede the headache, and that aura and headache are contralateral in attacks with unilateral symptoms. Acute onset aura, bilateral aura with unilateral headache, and ipsilateral aura and headache are real albeit rare phenomena.

Retrospective information. A random sample of 4000 forty years old participated in a screening questionnaire about migraine. The response rate was 87%. Persons with self-reported migraine were interviewed. The participation rate was 87%. Totally 484 migraineurs were included. The lifetime prevalence of MO was 14.7% with a male:female ratio of 1:2.2, and that of MA was 7.9% with a male:female ratio of 1:1.5. The age at onset of MO was unimodal and the age at onset of MA was bimodal. The female preponderance was present in all age groups in MA, but was first apparent after the menarche in MO, suggesting that female hormones are a confounding factor in MO, but not in MA. The expected and observed frequencies of co-occurrence of MO and MA were not significantly different. Menstruation was a precipitating factor in MO, but not in MA. Both MO and MA improved during pregnancy. Of the 163 persons with MA, 62 had co-occurrence of MA with and without headache and 7 had exclusively MA without headache. Visual aura was the most frequent symptom (98.8%), followed by sensory (31.3%), aphasic (17.8%) and motor (5.5%) aura. Persons with several types of aura only had sensory, motor and aphasic aura in a small number of their attacks, indicating that these types of aura are rare. The clinical differences indicate that MO and MA are distinct entities.

Lars Lykke Thomsen

Investigations into the role of nitric oxide and the large intracranial arteries in migraine headache

A doctoral thesis in medical science based on the following works:

Olesen J, Iversen HK, Thomsen LL. Nitric oxide supersensitivity: a possible molecular mechanism of migraine pain. *Neuroreport* 1993; 4; 1027–1030.

Thomsen LL, Kruuse C, Iversen HK, Olesen J. A nitric oxide donor (nitroglycerin) triggers genuine migraine attacks. *Eur J Neurol* 1994; 1: 73–80.

Thomsen LL, Iversen HK. Experimental and biological variation of three dimensional transcranial Doppler measurements. *J Appl Physiol* 1993; 75: 2805–2810.

Thomsen LL, Iversen HK, Brinck TA, Olesen J. Arterial supersensitivity to nitric oxide (nitroglycerin) in migraine sufferers. *Cephalalgia* 1993; 13: 395–399.

Thomsen LL, Daugaard D, Iversen HK, Olesen J. Normal radial artery dilatation during reactive hyperaemia in migraine without aura. *Endothelium.* 1996; 4: 199–206.

Thomsen LL, Iversen HK, Emmeluth C, Bie P. Venous plasma levels of endothelin-1 are not altered immediately after nitroglycerin infusion in healthy subjects. *Eur J Clin Pharmacol* 1995; 48: 139–142.

Thomsen LL, Iversen HK, Boesen F, Olesen J. Transcranial Doppler and cardiovascular responses during cardiovascular autonomic tests in migraineurs during and outside attacks. *Brain* 1995; 118: 1319–1327.

Thomsen LL, Brennum J, Iversen HK, Olesen J. Effect of a NO donor (glyceryl trinitrate) on nociceptive thresholds in man. *Cephalalgia* 1996; 16: 169–174.

Previous studies suggest that nitric oxide (NO) is involved in headaches induced by i.v. infusion of the vasodilator and NO donor glyceryl trinitrate (GTN) in healthy subjects. Extending these studies to sufferers of migraine without aura it was found that migraineurs experienced a stronger headache than non-migraineurs. In addition most migraineurs experienced a delayed migraine attack at variable times (mean 5.5 hours) after the GTN provocation. This biphasic headache response in migraineurs may be linked to hypersensitivity in the NO-cGMP pathway. Thus, compared to controls migraineurs were found to be more sensitive to GTN induced intracranial arterial dilatation, which is known to be mediated via liberation of NO and subsequent synthesis of cGMP. Furthermore, histamine infusions in migraineurs induced headache- and intracranial arterial-responses resembling those induced by GTN in migraineurs. Histamine is known to liberate NO from the endothelium via stimulation of the H_1 receptor. This receptor is present in the large intracranial arteries in man. Because both immediate histamine-induced –headache and -intracranial arterial dilation and delayed histamine-induced migraine are blocked by H_1-receptor blockade, a likely common pathway for GTN and histamine induced headaches/migraines and intracranial arterial responses may be via activation of the NO-cGMP pathway. The delay in the development of these experimental migraines may reflect activation of multiple physiological processes. In intracranial arteries migraineurs were found supersensitive to the vasodilating effect of GTN (exogenous NO). This relates to clinical findings suggesting dilatation of the large intracranial arteries on the headache side during spontaneous migraine attacks. The function of arterial regulatory mechanisms involving NO in migraine was therefore studied. In peripheral arteries no endothelial dysfunction of NO was found and cardiovascular and intracranial arterial sympathetic function was normal. A mild parasympathetic dysfunction may be involved and may, via

denervation supersensitivity, be responsible for the observed supersensitivity to NO. Another possibility is that NO initiates a perivascular neurogenic inflammation with liberation of vasoactive peptides. NO also mediates a variety of other physiological phenomena. One of these, the pain modulating effect observed in animals was evaluated in a human study using GTN infusion and measurements of pain thresholds. No definite effects of GTN were demonstrated. The precise mechanisms involved in NO triggered migraines and which part of the NO activated cascade that is involved remain to be determined. It is fascinating that the possibilities for pharmacological stimulation and/or inhibition of several steps of the NO activated cascade increase rapidly and soon may be available for human studies.

Lars Bendtsen

Exteroceptive suppression periods in tension-type headache

A PhD thesis based on the following works:
Bendtsen L, Jensen R, Brennum J, Arendt-Nielsen L, Olesen J. Exteroceptive suppression periods in jaw closing muscles. Variability and relation to experimental pain and sustained muscle contraction. *Cephalalgia* 1993; 13: 184–191.
Bendtsen L, Jensen R, Brennum J, Arendt-Nielsen L, Olesen J. Exteroceptive suppression of temporalis muscle activity is normal in patients with chronic tension-type headache and not related to actual headache state. *Cephalalgia* 1996; 16: 251–256.
Bendtsen L, Jensen R, Olesen J. Amitriptyline, a combined serotonin and noradrenaline re-uptake inhibitor, reduces exteroceptive suppression of temporal muscle activity in patients with chronic tension-type headache. *Electroencephalogr Clin Neurophysiol* 1996; 101: 418–422.

Tension-type headache is the most common and, as far as socioeconomic impact is concerned, the most important type of headache, but remarkably little is known about the pathophysiology of this disorder. It was therefore received with major interest when the duration of a brainstem reflex, the late exteroceptive suppression period (ES_2) of temporal muscle activity, in 1987 was reported to be reduced in patients with chronic tension-type headache. ES_2 is probably mediated via inhibitory brainstem interneurons and it has therefore been suggested that studies of ES_2 may provide information about the central mechanisms related to tension-type headache. However, the applied methodology, which is crucial for the reliability of ES_2 measurements, has been far from optimal in most previous ES_2 studies. The purpose of the present thesis was to develop a reliable method for measuring ES_2 and to apply this method in the study of ES_2 in patients with tension-type headache.

ES_2 was investigated in 30 healthy subjects, using a computerized technique of recording, rectifying and averaging the electromyographic signals from the temporal muscle. ES_2 durations varied considerably between subjects, while intra-individual variations from hour to hour and from week to week were acceptably low, and intra- and inter-observer variations were very low. It was concluded that the computerized averaging technique made it possible to measure ES_2 periods reliably. In a second blinded study, ES_2 was recorded in 55 patients with chronic tension-type headache and in 55 healthy controls. The duration of ES_2 did not differ between patients and controls and did not differ on days with headache compared with days without headache. It was concluded that ES_2 is normal in chronic tension-type headache and that the previous abnormal findings may have been method-dependent. Finally, ES_2 was recorded in 27 patients with chronic tension-type headache during treatment with amitriptyline, citalopram and placebo. Amitriptyline, a combined serotonin and noradrenaline re-uptake inhibitor, reduced ES_2 significantly, while citalopram, a selective serotonin re-uptake inhibitor, had no significant effect. These results suggest that amitriptyline modulates ES_2 via an effect on serotonergic as well as noradrenergic neurotransmission in the brainstem.

In conclusion, we have developed a reliable method for recording and analysing ES_2 and demonstrated that this brainstem reflex can be modulated by experimental pain and amitriptyline. Furthermore, we have demonstrated that ES_2 is normal in patients with chronic tension-type headache and that ES_2 is unrelated to a wide range of clinical and paraclinical parameters recorded in these patients. ES_2 therefore cannot be considered a useful tool for investigating the pathophysiology of tension-type headache.

Lars Lykke Thomsen

Transcranial Doppler examinations in relation to migraine pain

A PhD thesis based on the following works:
Thomsen LL, Iversen HK. CO_2 measurements during transcranial Doppler examinations in headache patients- methodological considerations. *Cephalalgia* 1994;14: 245–247.
Thomsen LL, Iversen HK, Olesen J. Cerebral blood flow velocities are reduced during attacks of unilateral migraine without aura. *Cephalalgia* 1995; 15: 109–116.
Thomsen LL, Iversen HK, Olesen J. Increased cerebrovascular pCO_2 reactivity in migrainewith aura – a transdermal Doppler study during hyperventilation. *Cephalalgia* 1995;15: 211–215.

It has been suspected for more than a century that cranial arteries are the locus of migraine pain. However, there has so far been little and conflicting scientific evidence to

support this. Unfortunately, diameters of intracranial arteries are difficult to measure in vivo. In situations of unchanged blood flow, the diameter of large intracranial arteries, can however, be estimated with the non-invasive ultrasound technique transcranial Doppler (TCD). This PhD thesis describes the principles of TCD and studies focusing on TCD responses in relation to migraine pain. A group of migraineurs suffering from attacks of unilateral migraine without aura were studied during and outside of attack. These patients were selected because cerebral blood flow most likely is unaffected during attacks of migraine without aura, and because a focus on side-to-side differences during unilateral headache provides optimal conditions for cross-over comparisons.

Furthermore, the TCD responses during a hyperventilation test was compared in migraineurs and non-migraineurs. Finally, TCD responses during experimental headache induced by i.v. infusion of glyceryl trinitrate (GTN) was examined. During migraine attacks evidence supporting a 9% increase in cross sectional vessel area of the middle cerebral artery (MCA) on the painful side was provided by the finding of ipsilateral decreased blood velocity during unilateral attacks of migraine without aura. This difference could not be explained by altered TCD responses during hyperventilation. The GTN-induced headache in healthy subjects showed similarities to migraine without aura but lacked the accompanying symptoms characteristic of migraine (nausea, vomiting, photo- and phonophobia). A decrease in MCA blood velocity to 80% of baseline confirmed previous studies applying TCD during GTN infusion and suggest that dilatation of intracranial large arteries also accompanies GTN induced headache. It is concluded that the diagnostic value of the systematic side-to-side blood velocity asymmetry found during attacks of migraine without aura, most likely is without any clinical.relevance due to the large albeit unsystematic side-to-side variation found in healthy subjects. Furthermore, that the magnitude of the intracranial artery dilatation may be too small to explain migraine headache on a purely mechanical basis. It may reflect a basic pathophysiological response, which simultaneously causes arterial dilatation and triggers sensory nerve fibers. Future TCD studies with newer technical possibilities in combination with highly specific pharmacological tools are likely to provide further answers.

Michael Langemark

Clinical studies of central and peripheral mechanisms

A doctoral thesis in medical science based on the following works:

Langemark M, Olesen J. Pericranial tenderness in tension headache. A blind, controlled study. *Cephalalgia* 1987; 7: 249–255.

Langemark M, Olesen J, Poulsen DL, Bech P. Clinical characterization of patients with chronic tension headache. *Headache* 1988; 28: 590–596.

Langemark M, Jensen K, Jensen TS, Olesen J. Pressure pain thresholds and thermal nociceptive thresholds in chronic tension-type headache. *Pain* 1989;38: 203–210.

Langemark M. Naloxone in moderate dose does not aggravate chronic tension headache. *Pain* 1989;39: 85–93.

Langemark M, Jensen K, Olesen J. Temporal muscle blood flow in chronic tension-type headache. *Arch Neurol* 1990; 47: 654–658.

Langemark M, Bach FW, Jensen TS, Olesen J. Decreased nociceptive flexion reflex threshold in chronic tension-type headache. *Arch Neurol* 1993; 50:1061–1064.

Langemark M, Bach FW, Ekman R, Olesen J. Increased cerebrospinal fluid Met-enkephalin immunoreactivity in patients with chronic tension-type headache. *Pain* 1995; 63:103–107.

Flemming W Bach

B-endorphin in cerebrospinal fluid: Relation to nociception

A doctoral thesis in medical science based on the following works:

Bach FW, Ekrnan R, Jensen FM. 13-endorphin -immunoreactive components in human cerebrospinal fluid. *Regulatory Peptides* 1986; 16: 189–198.

Bach FW, Schmidt J, Faber T. Radioimmunoassay of 13-endorphin in ventricular and lumbar cerebrospinal fluid. *Clinical Chemistry* 1992; 38: 847–852.

Bach FW, Langemark M, Secher NH, Olesen J. Plasma and cerebrospinal fluid 13-endorphin in chronic tension-type headache. *Pain* 1992; 5l: 163–168.

Young RF, Bach F, van Norman A, Yaksh TL. Release of 13-endorphin and met-enkephalin into cerebrospinal fluid during deep brain stimulation for chronic pain: effects of stimulation locus and site of sampling. *J Neurosurg* 1993; 79: 816–825.

Bach FW and Yaksh TL. Release into ventriculo-cisternal perfusate of beta-endorphin- and Met-enkephalin-immunoreactivity: effects of electrical stimulation in the arcuate nucleus and periaqueductal gray of the rat. *Brain Res* 1995; 690: 167–176.

Bach FW and Yaksh TL. Release of 13-endorphin immunoreactivity from brain by activation of a hypothalamic N-methyl-D aspartate-receptor. *Neuroscience* 1995; 65: 775–783.

Bach FW and Yaksh TL. Release of 13-endorphin immunoreactivity into ventriculo- cisternal perfusate by lumbar intrathecal capsaicin in the rat. *Brain Research* 1995; 701: 192–200.

Jannick Brennum

Quantitative sensory examination of epidural anaesthesia and analgesia in man

A doctoral thesis in medical science based on the following works:
Brennum J, Arendt-Nielsen L, Secher NH, Jensen TS, Ejerring P. Quantitative sensory examination in human epidural anesthesia and analgesia. Effects of lidocaine. *Pain* 1992; 51: 27–34.
Brennum J, Horn A, Arendt-Nielsen L, Secher NH, Jensen TS. Quantitative sensory examination during epidural anaesthesia and analgesia in man. Effects of morphine. *Pain* 1993; 52: 75–83.
Brennum J, Nielsen PT, Horn A, Secher NH, Arendt-Nielsen L. Quantitative sensory examinat ion during epidural anaesrhesia and analgesia in man. Dose-response effect of bupivacaine. *Pain* 1994; 56: 315–326.
Brennum J, Petersen KL, Horn A, Arend t-Nielsen L, Secher NH, J ensen TS, Quantitative sensory examinarion of epidural anaesthesia and analgesia in man. Combination of morphine and bupivacaine. *Pain* 1994; 56: 327–337.
Brennum J, Dahl JB, Møiniche S, Arendt-Nielsen L. Quantitative sensory examinati on of epidural anaesthesia and analgesia in man. Effects of preemptive and posttraumatic morphine on hyperalgesia. *Pain* 1994; 59: 261–271.

Lisbeth Hjorth Lassen

Histamine induced headache and migraine. Possible involvement of nitric oxide

A PhD thesis based on the following works:
Lassen LH, Thomsen LL, Olesen J. Histamine induces migraine via H_1 -receptor activation. Support for the NO hypothesis of migraine. *Neuro Report.*1995; 6: 1475–1479.
Lassen LH, Thomsen LL, Kruuse C, Iversen HK, Olesen J. Histamine-l receptor blockade does not prevent nitroglycerin induced migraine. Support for the NO hypothesis of migraine. *Eur J Clin Pharrnacol* 1996; 5: 335–341.
Lassen LH, Heinig JH, Østergård S, Olesen J. Histamine inhalation is a specific but insensitive laboratory test for migraine. *Cephalalgia* 1997; 16: 550–553.

It has previously been shown that in migraine sufferers infusion of the exogenous nitric oxide (NO)- donor glyceryl trinitrate (GTN) causes an immediate headache during the infusion and a genuine migraine attack with peak headache intensity 5.5 hours after the infusion. Now histamine induced headache is evaluated. In primates histamine activates cerebral endothelial HI-receptors leading to formation of NO. Twenty migraine patients received pretreatment with placebo or the histamine- H_1-receptor antagonist, mepyramine, in a randomized, double blind fashion, followed in both groups by intravenous histamine 0.5 p.g/kg/min for 20 minutes. Headache characteristics were subsequently observed for 12 hours. Blood velocity in the middle cerebral artery was recorded during the first 3 hours. In placebo pretreated patients, histamine caused the previously described immediate headache during the infusion.

Furthermore, it was demonstrated that it causes a delayed headache attack fulfilling the IHS criteria for migraine without aura, with peak headache intensity 5.l hours from start of histamine infusion. Histamine reduced blood velocity in the middle cerebral artery probably due to arterial dilatation. In the mepyramine pretreated group the effect on the blood velocity in the middle cerebral artery and the immediate headache was greatly reduced and the delayed migraine attacks were abolished. Since intravenous histamine does not cross the blood brain barrier, and H_1 receptors are predominating in cerebral contra extracranial arteries, the present study suggests that migraine attacks can be elicited from the endothelium of cerebral arteries. Furthermore, the temporal profiles of histamine induced headache and arterial dilatation were exactly the same as previously observed after GTN, indicating a common mechanism of action. This mechanism most likely involves NO.

Others have proposed that GTN causes headache via liberation of histamine from perivascular mast cells. To evaluate this, the effect of intravenous GTN (0.5 μg/kg/min) for 20 minutes in 7 migraine sufferers, once after pretreatment with the histamine H_1-receptor antagonist, mepyramine (0.5 mg/kg) and once without pretreatment were studied.

After mepyramine 5 patients developed migraine, and without pretreatment 6 patients did so ($p = 0.317$). The median peak headache score was 7 on a 0–10 scale with and without mepyramine pretreatment, ($p = 0.89$). The arterial responses, evaluated with transcranial Doppler, were also unaffected by the mepyramine pretreatment. These results demonstrate that neither headache nor arterial dilatation with GTN infusion is caused by histamine release. It is most likely that both GTN and histamine triggers migraine attacks via NO.

In the last study it was evaluated if histamine inhalation may be used as a diagnostic test for migraine. Migraine is a subjective complaint and no laboratory test has until now been of value. In a double blind study design, 15 migraineurs and 15 control subjects scored headache intensity and characteristics before, during, and in the subsequent 12 hours after inhalation of increasing doses of histamine (0,2,4,8,16,32 and 64 mg/ml). During the histamine inhalations, headaches increased dose dependently in both groups. Eleven of the migraineurs and 7 of the healthy controls experienced headaches after the inhalations. These headaches fulfilled the IHS criteria for migraine

without aura in six of the migraineurs, but in none of the control subjects. Using this as a test parameter, the specificity of the test was 1, but the sensitivity only 0.4. Our results indicate that histamine inhalation is a specific but insensitive laboratory test for migraine. Migraineurs should be informed about the risk of provoking a migraine attack before histamine inhalation in pulmonary laboratories.

Vibeke Ulrich

The inheritance of migraine with aura investigated by analyses of twins

A PhD thesis based on the following works:
Ulrich V, Gervil M, Fenger K, Olesen J, Russell MB. The prevalence and characteristics of migraine in twins from the general population. *Headache* 1999; 39: 173–180.
Ulrich V, Gervil M, Kyvik KO, Olesen J, Russell MB. Evidence of a genetic factor in migraine with aura: a population-based Danish twin study. *Ann Neurol.* 1999; 45: 242–246.
Ulrich V, Gervil M, Kyvik KO, Olesen J, Russell MB. The inheritance of migraine with aura estimated by means of structural equation modelling. *J Med Genet.* 1999; 36: 225–227.

The aim of the PhD thesis was to investigate the relative strength of the genetic and environmental effects in migraine with aura (MA), and to estimate the degree of heritability of MA. The study was made by analyses of twins, a classic way to study the importance of genetic and environmental effects in etiology of diseases due to differences in concordance among monozygotic (MZ) and dizygotic (DZ) twin pairs. The twin sample was from the population-based New Danish Twin Register, and consisted 5360 twins (2026 MZ, 3334 same sex DZ) born 1953–60. In 1994 87% (4660/5360) of the twins had completed a questionnaire screening for migraine and migraine symptoms. Twin pairs with at least one twin selfreporting migraine or migraine symptoms were contacted by telephone and interviewed by a physician about migraine. The criteria of the International Headache Society were used for diagnosis. The participation rate of the telephone interview was 90% (2035/2272). MA was identified in 264 twins (118 males, 146 females). The lifetime prevalence of MA was 7%, and similar in MZ and DZ twins with a male to female ratio of 1: 1.1. Equivalent lifetime prevalence of MA was found in two recent Danish non-twin population-based studies. Thus, status as a twin did not affect the lifetime prevalence of MA. Twenty-six of 77 MZ twin pairs (34%) and 16 of 134 DZ twin pairs (12%) were concordant for MA, which was a significant difference and emphasized the importance of genetic factors in MA. Correspondingly, the risk of MA in the co-twin of an affected twin was higher in MZ (50%) than in DZ twins (21%).

The concordance rate of less than 100% in MZ twin pairs indicates the importance of environmental factors in MA. Multifactorial inheritance was considered most likely in MA.

By structural equation modelling, different combinations of genetic effects (additive or non additive) and effects of environment (shared or non-shared) were evaluated. The best-fitting model combined additive genetic effects and effects in environment that were non-shared by the twin pairs. The estimate of heritability was 0.65 under the best-fitting model.

Michael Bjørn Russel

Genetic epidemiology of migraine and cluster headache

A doctoral thesis in medical science based on the following works:
Russell MB, Hilden J, Sørensen SA, Olesen J. Familial occurrence of migraine without aura and migraine with aura. *Neurology* 1993; 43: 1369–1373.
Russell MB, Rasmussen BK, Thorvaldsen P, Olesen J. Prevalence and sex-ratio of the subtypes of migraine. *Int J Epidemio/1995;* 24: 612–618.
Russell MB, Olesen J. Increased familial risk and evidence of a genetic factor in migraine. *BMJ* 1995; 311: 541–544.
Russell MB, Fenger K, Olesen J. The family history of migraine. Direct versus indirect information. *Cephalalgia* 1996; 16: 156–160.
Russell MB, Iselius L, Olesen J. Inheritance of migraine investigated by complex segregation analysis. *Hum Genet* 1995; 96: 726–730.
Russell MB, Andersson PG, Thomsen LL. Familial occurrence of cluster headache. *J Neurol Neurosurg Psychiatry* 1995; 58: 341–343.
Russell MB, Andersson PG. Clinical intra- and interfamilial variability of cluster headache. *Eur J Neurol 1995;* 1: 253–257.
Russell MB, Andersson PG, Thomsen LL. Iselius L. Cluster headache is an autosomal dominant inherited disorder in some families. A complex segregation analysis. *J Med Genet* 1995; 32: 954–956.

Migraine. The present genetic epidemiological survey of migraine was based on semi structured interviews by a physician. The operational diagnostic criteria of the International Headache Society were used. Three thousand males and one thousand females aged 40 were drawn from the Danish National Central Person Registry. They received a posted questionnaire regarding migraine. The questionnaire response rate was 87%. People with self-reported migraine and a random sample of people reporting no migraine were invited to a

headache interview, and a physical and a neurological examination. Those not reacting to the invitation were interviewed by telephone.The participation of the interview was 87%. Kappa was 0.77 validating self-reported migraine against a clinical interview by a physician. Non-responders did not differ from responders regarding migraine. The lifetime prevalence of MO and MA was 9.3% and 6.3% in males and 20.1% and 9.6% in females. People with MA were included as probands in the family study. An equivalent number of probands with MO and probands who had never had migraine were random samples of the people with MO and the people who had never had migraine, respectively. First degree relatives and spouses were interviewed blindly by a physician.The 378 proband s had 1109 first degree relatives and 229 spouses. Compared with the general population, the first degree relatives of probands with MO had a 1.9-fold increased risk of MO and a 1.4-fold increased risk of MA, after standardization for sex and age. The first degree relatives of probands with MA had a 3.8-fold increased risk of MA and no increased risk of MO. The first degree relatives of probands who had never had migraine had no increased risk of neither MO nor MA. Spouses to probands with MO had a 1.5-fold increased risk of MO, while spouses to proba.nds with MA had no increased risk of MA. The familial patterns indicate that MO and MA are distinct entities. The familial occurrence suggests that MO is caused by a combination of genetic and environmental factors, while MA is determined mainly or exclusively by genetic factors. The complex segregation analysis supports the importance of genetic factors and suggests multifactorial inheritance without generational difference in both MO and MA, but genetic heterogeneity cannot be excluded.

Conclusion. MO and MA are distinct entities. Both MO and MA are multi-factorial inherited without generational differences.

Cluster headache. The inheritance of cluster headache was investigated in 421 probands from a neurological clinic and two departments of neurology.

The operational diagnostic criteria of the International Headache Society were used. The probands received a posted questionnaire regarding the presence of duster headache among relatives. The questionnaire response rate was 88%. Possibly affected relatives were interviewed by telephone by a physician. A positive family history of duster headache was found in 7% of the families. Compared with the general population, first degree relatives of probands with cluster headache had a 14-fold increased risk of cluster headache, after standardization for sex and age. Second degree relatives had a 2- fold increased risk of cluster head ache. The significantly increased familial risk of cluster headache strongly suggests a genetic cause. An analysis of the clinical intra- and inter-familial variability of duster headache was not indicative of genetic heterogeneity. The complex segregation analysis suggests that an autosomal dominant gene has a role in some families with duster headache.

Conclusion. Cluster headache is an autosomal dominant inherited disorder in some families.

Rigmor Jensen

Pathophysiological mechanisms of tension-type headache

A doctoral thesis in medical science based on the following works:

Jensen R, Fuglsang-Frederiksen A, Olesen J. Quantitative surface EMG of the pericranial muscles. Reproducibility and variability. *Electroencephalogr Clin Neurophysiol* 1993; 89: 1–9.

Jensen R, Rasmussen BK, Pedersen B, Lous I, Olesen J. Cephalic muscle tenderness and pressure pain threshold in a general. population. *Pain* 1992;48: 197–203.

Jensen R, Rasmussen BK, Pedersen B, Olesen J. Muscle tenderness and pressure pain threshold in headache. A population study. *Pain* 1993; 52: 193–199.

Jensen R, Fuglsang-Frederiksen A. Quantitative surface EMG of pericranial muscles. Relation to age and sex in a general population. *Electroencephalogr Clin Neurophysiol* 1994; 93: 175–183.

Jensen R, Fuglsang-Frederiksen A, Olesen J. Quantitative surface EMG in headache. A population study. *Electroencephalogr Clin Neurophysiol* 1994; 93: 335–344.

Jensen R, Rasmussen BK. Muscular disorders in tension-type headache. *Cephalalgia* 1996; 16: 97–103.

Jensen R. Mechanisms of spontaneous tension-type headaches: an analysis of tenderness, pain thresholds and EMG. *Pain*1996; 64: 251–256.

Jensen R, Olesen J. Initiating mechanisms of experimentally induced tension-type headache. *Cephalalgia* 1996; 16: 175–182.

The present thesis discusses the epidemiology and possible pathophysiological mechanisms of tension-type headache. A population based study of 1000 subjects randomly selected from a general population, 2 clinical studies and a method study of EMG recordings, were conducted. Tension-type headache was the most prevalent form of headache with a life time prevalence of 78% in a general adult population. Thirty per cent were affected more than 14 days per year and 3 per cent were chronically affected, i.e. had headache at least every other day. Females were more frequently affected than males, and young subjects more frequently affected than older subjects. Females were more sensitive to mechanical pressure pain and revealed more tenderness from pericranial muscles and tendon insertions than males, and young sub jects were more pain sensitive than older subjects. Significantly higher tenderness in pericranial muscles was found in subjects

with tension-type headache compared to migraineurs and to subjects without any experience of headache. Tenderness increased significantly with increasing frequency of tension-type headache in both males and females whereas no such relation was found for mechanical pain thresholds. The applied EMG methodology was fairly reliable and non-painful, but due to inter-subject variability paired studies should be preferred. Subjects with chronic tension-type headache had slightly increased EMG levels during resting conditions and decreased levels during maximal voluntary contraction compared with headache free subjects indicating insufficient relaxation at rest and impaired recruitment at maximal activity. In a subsequent clinical, controlled study, the effect of 30 minutes of sustained tooth clenching was studied. Within 24 hours 69% of patients and 17% of controls developed a tension-type headache. Shortly after clenching, tenderness was increased in the group who subsequently developed headache, whereas tenderness was stable in the group of patients who remained headache free indicating that tenderness might be a causative factor to the headache. Likewise, psychophysical and EMG parameters were studied in 28 patients with tension-type headache both during and outside of a spontaneous episode of tension-type headache. It was concluded that a peripheral mechanism of tension-type headache is most likely in the episodic subform, whereas a secondary, segmental central sensitization and/or an impaired supraspinal modulation of incoming stimuli seem to be involved in subjects with chronic tension-type headache. Prolonged nociceptive stimuli from myofascial tissue may be of importance for the conversion of the episodic into the chronic tension-type headache. The author emphasizes that tension-type headache is a multifactorial disorder with several concurrent pathophysiological mechanisms, and that extracranial myofascial nociception may constitute only one of them. The present thesis supplements the understanding of the balance between peripheral and central components in tension-type headache, and thereby, hopefully, lead us to a better prevention and treatment of the most prevalent type of headache.

Lars Bendtsen

Central sensitization in tension-type headache

A doctoral thesis in medical science based on the following works:

Bendtsen L, Jensen R, Jensen NK, Olesen J. Muscle palpation with controlled finger pressure: new equipment for the study of tender myofascial tissues. Pain 1994;59:235–239.

Bendtsen L, Jensen R, Jensen NK, Olesen J. Pressure-controlled palpation: a new technique which increases the reliability of manual palpation. *Cephalalgia* 1995; 15: 205–210.

Bendtsen L, Jensen R, Olesen J. Decreased pain thresholds and tolerances in chronic tension-type headache. *ArchNeurol* 1996; 53: 373–376.

Bendtsen L, Jensen R, Olesen J. Qualitatively altered nociception in chronic myofascial pain. *Pain* 1996; 65: 259–264.

Bendtsen L, Nørregaard J, Jensen R, Olesen J. Evidence of qualitatively altered nociception in patients with fibromyalgia. *Arthritis and Rheumatism* 1997; 40: 98–102.

Bendtsen L, Jensen R, Olesen J. A non-selective (amitriptyline), but not a selective (citalopram), serotonin reuptake inhibitor is effective in the prophylactic treatment of chronic tension-type headache. *J Neurol Neurosurg Psychiatry* 1996; 61: 285–290.

Bendtsen L, Jensen R, Hindberg I, Gammeltoft S, Olesen J. Serotonin metabolism in chronic tension-type headache. *Cephalalgia* 1997; 17: 843–848.

Bendtsen L, Mellerup ET. The platelet serotonin transporter in primary headaches. *Eur J Neurol* 1998; 5: 277–282.

The aim of the present thesis was to investigate the pathophysiology of chronic tension-type headache with special reference to central mechanisms. Increased tenderness to palpation of pericranial myofascial tissues is the most apparent abnormality in patients with tension-type headache. Two methodological studies concerning manual palpation were therefore conducted. In the first study, a new instrument, a so-called palpometer, that makes it possible to control the pressure intensity exerted during palpation was developed. In the second study, it was demonstrated that the measurement of tenderness could be compared between two observers if the palpation pressure was controlled, and that the Total Tenderness Scoring system was well suited for the scoring of tenderness during manual palpation. Subsequently, pain sensitivity was studied in 40 patients with chronic tension type headache and in 40 healthy controls. Pressure pain detection and tolerance thresholds were significantly decreased in the finger and tended to be decreased in the temporal region in patients compared with controls. In addition, the electrical pain threshold in the cephalic region was significantly decreased in patients. It was concluded that the central pain sensitivity was increased in the patients, probably due to sensitization of supraspinal neurons. The stimulus-response function for palpation pressure versus pain was found to be qualitatively altered in chronic tension-type headache patients compared with controls. The abnormality was related to the degree of tender ness and not to the diagnosis of tension-type headache. In support for this, the stimulus-response function was found to be qualitatively altered also in 25 women with fibromyalgia. It was concluded that the qualitatively altered nociception most likely was due to central sensitization at the level of the spinal dorsal horn/trigeminal nucleus. Thereafter, the prophylactic effect of amitriptyline, a non-selective serotonin (5-HT)

reuptake inhibitor, and of citalopram, a highly selective 5-HT reuptake inhibitor, was examined in 40 patients with chronic tension-type headache. Amitriptyline reduced headache significantly more than placebo, while citalopram had only a slight and insignificant effect. It was concluded that the blockade of 5-HT reuptake could only partly explain the efficacy of amitriptyline in tension-type headache, and that also other of the actions of amitriptyline, e.g. reduction of central sensitization, were involved. Finally, the plasma 5-HT level, the platelet

5-HT level and the number of platelet 5-HT transporters were found to be normal in patients with chronic tension-type headache compared with healthy controls. On the basis of the present and previous studies, a pathophysiological model for chronic tension-type headache is presented. According to the model, the main problem in chronic tension-type headache is central sensitization at the level of the spinal dorsal horn/trigeminal nucleus due to prolonged nociceptive inputs from pericranial myofascial tissues. The increased nociceptive input to supraspinal structures may in turn result in supraspinal sensitization. The central neuroplastic changes may affect the regulation of peripheral mechanisms and thereby lead to, e.g. increased pericranial muscle activity or release of neurotransmitters in the myofascial tissues. By such mechanisms the central sensitization may be maintained even after the initial eliciting factors have been normalized, resulting in the conversion of episodic into chronic tension-type headache. Future basic and clinical research should aim at identifying the source of peripheral nociception in order to prevent the development of central sensitization and at ways of reducing established sensitization. This may lead to a much needed improvement in the treatment of chronic tension-type headache and other chronic myofascial pain conditions.

Messoud Ashina

Muscle hardness in chronic tensions-type headache

A PhD thesis based on the following works:
Ashina M, Bendtsen L, Jensen R, Sakai F, Olesen J. Measurement of muscle hardness: a methodological study. *Cephalalgia* 1998; 18: 106–111.
Ashina M, Bendtsen L, Jensen R, Sakai F, Olesen J. Muscle hardness in patients with chronic tension-type headache: relation to actual headache state. *Pain* 1999; 79: 201–205.
Ashina M, Bendtsen L, Jensen R, Sakai F, Olesen J. Possible mechanisms of glyceryl-trinitrate-induced immediate headache in patients with chronic tension-type headache. *Cephalalgia* 2000; 20(10): 919–924.

Increased tenderness to palpation of pericranial myofascial tissues is the most prominent finding in patients with chronic tension-type headache. During manual palpation it is a common clinical experience that tender muscles are harder, i.e., they have a higher consistency than normal muscles. Until 1995 no quantitative method to make an objective evaluation of muscle hardness had been used in patients with myofascial pain conditions. It was, therefore, received with major interest when Japanese researchers developed a new device for quantitative and non-invasive measurement of muscle hardness, the so-called hardness meter. Using the hardness meter, they demonstrated that pericranial muscles in patients with chronic tension-type headache are significantly harder than in healthy subjects. The aim of the present thesis is to evaluate a method for recording muscle hardness and to apply this method in the study of myofascial factors in chronic tension-type headache. Intra- and inter-observer variation during measurement of muscle hardness with the hardness meter was determined in 20 volunteers. In addition, we investigated the factors which may influence the recording of muscle hardness and whether the hardness differs within the same muscle. The intra-observer variation was 10% and the inter-observer variation was 12% for recording of muscle hardness. It was concluded that the hardness meter can measure muscle hardness reliably if the same observer is used throughout a study. The speed of applied pressure was a major reason of variability between observers, and muscle hardness differed within the same muscle. In the second study, hardness of the trapezius muscle and myofascial tenderness of pericranial muscles were recorded in 20 patients with chronic tension-type headache and in 20 healthy controls. The patients were examined on two days, one day with headache and the other day without headache. It was concluded that muscle hardness and muscle tenderness are permanently increased in chronic tension-type headache and not only a consequence of the actual pain episode, and that that increased muscle hardness is significantly correlated to increased local tenderness. Finally, using the hardness meter, the role of myofascial factors during nitric oxide (NO)-induced immediate headache was investigated in double-blind, placebo controlled, crossover study in 16 patients with chronic tension-type headache and in 16 healthy controls. Infusion of NO donor, glyceryl trinitrate, did not cause any significant change in muscle hardness or myofascial tenderness. It was concluded that myofascial factors do not contribute to NO-induced immediate headache, and that the immediate headache may be caused by increased nociceptive input from the cranial vasculature. In conclusion, the hardness meter can measure muscle hardness reliably and muscle hardness is permanently increased in patients with chronic tension-type headache. Furthermore, the results of the thesis indicate that the hardness meter will be an important tool to study the myofascial factors in various clinical and experimental pain models.

Christina Kruuse

Distribution and function of cyclic nucleotide phosphodiesterases (PDE's) in cerebral arteries

A PhD thesis based on the following works:
Kruuse C, Jacobsen TB, Lassen LH, Thomsen LL, Hasselbalch SG, Dige-Petersen H, et al. Dipyridamole dilates large cerebral arteries concomitant to headache induction in healthy subjects. *J. Cereb. Blood Flow Metab*. 2000; 20: 1372–1379.
Kruuse C, Jacobsen TB, Thomsen LL, Hasselbalch SG, Frandsen EK, Dige-Petersen H, et al. Effects of the non-selective phosphodiesterase inhibitor pentoxifylline on regional cerebral blood flow and large arteries in healthy subjects. *Eur J Neurol* 2000; 7: 629–638.
Kruuse C, Rybalkin SD, Khurana TS, Jansen-Olesen I, Olesen J, Edvinsson L. The role of cGMP hydrolysing phosphodiesterases 1 and 5 in cerebral artery dilatation. *Eur. J. Pharmacol.* 2001; 420: 55–65.

It was the aim to study the possible role of cyclic nucleotide phosphodiesterases (PDE's) in relation to the cGMP pathway in guinea pig basilar arteries and in human cerebral arteries in vitro. Furthermore, the effects of a non-selective PDE inhibitor pentoxifylline and the inhibitor of the cGMP degrading PDE5, dipyridamole on cerebral circulation were investigated in healthy subjects.

PDE's are intracellular enzymes responsible for degradation of the second messengers, cGMP and cAMP, and thus play a key role in, for instance, cGMP dependent effects in arterial responses. Their distribution and physiological effects varies between tissue and vascular beds which makes PDE's excellent targets for pharmacological modulation by selective drugs, when the distribution is known. In cerebral arteries, the distribution and functions of PDE's are not fully clarified.

In vitro experiments revealed the presence and activity of the cGMP degrading PDE1 and PDE5 in guinea pig basilar arteries and only minor cAMP degrading activity. Inhibition of PDE1 and PDE5 elicited an almost similar endothelium dependent dilatory response, which was decreased when cGMP production was inhibited. A part of the relaxant response to PDE5 inhibitors used seemed however, to be independent of cGMP concentrations, probably due to a lack of selectivity of the PDE5 inhibitors used. In isolated human cerebral arteries, only a minor dilatory response to pentoxifylline was seen, however a PDE5 inhibitor induced dilatation, which was almost equal to that seen in guinea pig cerebral arteries. Pentoxifylline showed no dilating effect on cerebral arteries in healthy subjects compared to placebo, although an increase in cAMP but not cGMP was seen. Dipyridamole caused a modest pCO_2 independent dilatation of the middle cerebral artery. Investigations both in healthy subjects and in patients using selective PDE1 or PDE5 inhibitors will perhaps reveal more of the cGMP mechanisms involved in regulation of cerebral artery dilatation. PDE inhibitors may thus be promising tools to elucidate possible vascular disease mechanisms and new aspects of specific treatment of disease with altered cerebral arterial response.

Morten Gervil

A genetic-epidemiological investigation of migraine without aura. A population-based twin study

A PhD thesis based on the following works:

The present PhD thesis was a twin study with the purpose of investigating the relative influence of genetic and environmental factors in the etiology of migraine without aura (MO). The study population was from the population-based Danish Twin Register and consisted of 2,680 twin pairs (1,420 male and 1,260 female pairs) born 1953–60. "Twin omnibus 94", a large questionnaire send to all twins in the Danish Twin Register, included four questions screening for migraine. In 87% (2,330/2,680) of the twin pairs one or both of the twin answered one or more of the screening questions. Twin pair s with at least one twin answering yes to at least one of the screening questions had a diagnostic telephone interview by a physician. MO was diagnosed according to the criteria of the International Headache Society. The participation in the telephone interview was 90% (1,01711,136). The screening questionnaire had an estimated sensitivity of 85% and specificity of 81%. The predictive positive value was 86% and the predictive negative value was 49%. Lifetime prevalence of MO was 18% with a male – female ratio of 1: 3. Similar lifetime prevalence of MO was found in previous Danish population -based survey in non-twin populations. Therefore, being a twin did not affect the lifetime prevalence of MO. 394 twin pairs with one or both twins having MO were identified. The pairwise concordance rate was 28 for monozygotic twin pairs and 18% for dizygotic twin pairs ($p = 0.04$). Correspondingly, the risk of MO in the co-twin of an affected twin (the proband wise concordance rate) was higher in MZ (40%) than in DZ (28%) twins. Assuming multifactorial inheritance biometric model-fitting showed a significant genetic influence in MO. However, environmental factors were of almost equal importance. The best fitting model estimated that 61% (C.I. 0.49–0.71) of the liability to MO was due to additive genetic effects and 39% (C.I. 0.29–0.51) was due to non-shared environmental effects. In order to explore the genetic load of MO, parents and siblings of 63 MZ twin pairs concordant and discordant for MO were blindly telephone interviewed by a physician. Parents and siblings of MZ twin pairs concordant for MO tended to have a higher risk of MO than parents and sibling of MZ twin pairs discordant for MO, although this difference did not attain statistical significance. However, the result indicated that the genetic load might be higher in concordant than in discordant MZ twin pairs.

An analysis often symptomatology of MO in the twin sample found a significantly higher concordance rate for pain characteristics and accompanying symptoms in MZ twin pairs than in DZ twin pairs. This indicated that genetic factors might be involved in the symptomatology of MO. The concordance rate for the presence or absence of precipitating factors was similar in MZ and DZ twin pairs. This may be caused by a lack of specificity of the precipitating factors.

To conclude, this study found a significant genetic factor in the etiology of MO. However, environmental factors were of equal importance. The results indicate that the model of genetic transmission is most likely a multifactorial threshold model (MFT). Under an MFT model, genetic factors are assumed to be polygenic. That is, there are a large number of genes, each of small and equal effect that combine additively with the effects of other genes and environmental factors to influence MO liability. Thus, the role of genetic factors may be complex and the identification of susceptibility genes is predicted to be difficult.

Although this difference did not attain statistical significance. However, the result indicated that the genetic load might be higher in concordant than in discordant MZ twin pairs.

An analysis of the symptomatology of MO in the twin sample found a significantly higher concordance rate for pain characteristics and accompanying symptoms in MZ twin pairs than in DZ twin pairs. This indicated that genetic factors might be involved in the symptomatology of MO. The concordance rate for the presence or absence of precipitating factors was similar in MZ and DZ twin pairs. This may be caused by a lack of specificity of the precipitating factors.

To conclude, this study found a significant genetic factor in the etiology of MO. However, environmental factors were of equal importance. The results indicate that the model of genetic transmission is most likely a multifactorial threshold model (MFT). Under an MFT model, genetic factors are assumed to be polygenic. That is, there are a large number of genes, each of small and equal effect that combine additively with the effects of other genes and environmental factors to influence MO liability. Thus, the role of genetic factors may be complex and the identification of susceptibility genes is predicted to be difficult.

Helle Klingenberg Iversen

Glyceryl trinitrate-induced headache: an experimental model of headache in humans

A doctoral thesis in medical science based on the following works:

Iversen HK, Olesen J, Tfelt-Hansen P. Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics. *Pain* 1989; 38:17–24.

Iversen HK, Nielsen TH, Garre K, Tfelt-Hansen P, Olesen J. Dose- dependent headache response and dilatation of limb and extracranial arteries after three doses of 5-isosorbidemononitrate. *Eur J Clin Pharmacol* 1992; 42: 31–35.

Iversen HK. N-acetylcysteine enhances nitroglycerin-induced headache and cranial arterial responses. *Clin Pharmacol Ther* 1992; 52:125–133.

Iversen HK, Nielsen TH, Tfelt-Hansen P, Olesen J. Lack of tolerance of headache and radial artery diameter during a 7 hour intravenous infusion of nitroglycerin. *Eur J Clin Pharmacol* 1993; 44: 47–50.

Migraine is often regarded as a mild condition; however, the disease has a considerable influence on quality of live. Along with the fast development of biomedicine, the need for experimental models has increased. The optimal model is an animal model; however, no animal model of migraine exists. Genuine migraine attacks are difficult to study because of their attack-wise appearance. We therefore aimed to develop a set-up for human experimental headaches, giving the possibility to produce headache under safe and controlled conditions. Glyceryl trinitrate (GTN) was selected as headache inducing agent, as the effects are well known and it is believed that GTN is metabolised to nitric oxide (NO) and/or S-nitrosothiols. The GTN-induced headache was described in healthy subjects with no history of migraine and who seldom ($<$1/month) or never suffered from headache. Nine of 10 subjects experienced headache after i.v. GTN. Headache intensity was mild to moderate and maximum intensity was reached within few minutes and declined rapidly after termination of GTN infusions. A retest was performed after at least one week and the day-to-day variation in headache intensity and characteristics were acceptable. The induced headache fulfilled the pain criteria for migraine without aura, but there were no accompanying symptoms, which must be present if the headache should fulfil the migraine criteria. It would be practical if two or more GTN infusions could be given to one subject on one study day, however, studies of repetitive infusions showed that 20 minutes wash-out periods were too short. An interval of at least 2 days between GTN infusions is recommended. A reproducible ceiling effect in headache score at 0.5 μg/kg/min was found from the dose-response curves. To follow GTN activity on the arteries, diameters of the temporal and radial arteries were measured using high frequency ultrasound. The GTN induced dilatation of temporal and radial arteries showed a similar time-profile and ceiling effect as the GTN-induced headache. This was not due to tolerance to GTN, as 7 hours GTN infusion showed no attenuation of the GTN-induced headache and dilatation of the radial artery.

The mechanisms were then investigated and it was concluded that NO or other parts downstream in the

activated NO-cGMP cascade were responsible for the induced headache, because:
- The long-acting nitrate, 5-isosorbide-mononitrate (5-ISMN) gives rise to NO formation but its other metabolites are different from those of GTN. A close relation between 5-ISMN plasma levels, headache and arterial dilatation was found after 15 -, 30 -, 60 mg 5-ISMN and placebo. Therefore, GTN-induced headache must be induced by the action of NO, and not by its other metabolites.
- N-acetylcysteine (NAC) enhances the cardiac vasodilatory effects of GTN, presumably by stimulating the NO production. NAC also potentiated the GTN-induced headache and dilatation of the temporal artery.
- The GTN-induced headache could be secondary to GTN-induced histamine release. Pre-treatment with mepyramine, a H_1 antagonist able to block histamine-induced headache, did not change the GTN-induced headache and arterial responses. Histamine causes release of NO via endothelial H_1 receptors, and NO may therefore be the mediator in both GTN- and histamine-induced headache.

The value of the GTN-model in migraine drug development is dependent on the response to migraine specific drugs and the power of the model. The last study showed that sumatriptan, the first selective $5HT_{1B/1D}$ agonist highly effective in treating migraine attacks, significantly decreased the GTN-induced headache. The study demonstrated that headache relief could be detected in a small sample size and that the immediate GTN-induced headache must share some mechanisms with migraine. Other studies using the presented set-up showed that GTN induces migraine attacks in migraine patients and tension-type headache in patients with chronic tension-type headache. In cluster headache patients GTN induces cluster attacks but only during a cluster period. The set-up offers the possibility to study all phases of the headache attack and to explore pathophysiology by comparing induced responses in patients and healthy subjects. At last, use of the model enables a more direct interaction between human and animal studies. Increased knowledge about differences due to species, topography and circumstances will advance basic understanding and make it easier to progress into focused research areas.

Hanne Mørk Christensen

A new experimental human model of myofascial tenderness and pain

A PhD thesis based on the following works:
Mørk H, Ashina M, Bendtsen L, Olesen J, Jensen R. Experimental muscle pain and tenderness following infusion of endogenous substances in humans. *Eur J Pain* 2003; 7: 145–153.
Mørk H, Ashina M, Bendtsen L, Olesen J, Jensen R. Possible mechanisms of pain perception in patients with episodic tension-type headache. A new experimental model of myofascial pain. *Cephalalgia* 2004; 24: 466–475.
Mørk H, Ashina M, Bendtsen L, Olesen J, Jensen R. Induction of prolonged tenderness in patients with tension-type headache by means of a new experimental model of myofascial pain. *Eur J Neurol.* 2003 May; 10: 249–256.

Increased tenderness to palpation of pericranial myofascial tissues is the most prominent abnormal finding in patients with tension-type headache. Numerous animal models of myofascial pain and tenderness have demonstrated that the release of various endogenous substances may excite or sensitize myofascial nociceptors, or both. The first aim of the present thesis was to develop a clinically relevant model of human myofascial pain using infusions into the trapezius muscle of the endogenous substances bradykinin (Bk), serotonin (5-hydroxytryptamine (5-HT)), histamine (His), prostaglandin E_2 (PGE_2), adenosine-triphosphate (ATP), and their combinations. Secondly, the developed model was applied to patients with episodic tension-type headache (ETTH) to elucidate possible pathophysiological factors of tension -type headache. In an open labelled design 36 healthy subjects participated in a total of 67 sessions to identify single substances or combinations of substances that provoked a pain of moderate intensity. PGE_2, ATP and a combination of Bk, 5-HT, Histamine, and PGE_2 were further investigated in a randomized, double blinded and placebo -controlled trial. Fifteen healthy subjects participated in a total of 68 sessions.

ATP and the combination provoked the intended reversible pain and tenderness of moderate intensity and prolonged duration. Unfortunately, ATP produced unacceptable side effects in subsequent examinations and further studies with ATP were therefore suspended.

The model using a combination of endogenous substances was applied to 15 patients with ETTH and 15 sex- and age- matched healthy controls in a randomized, double-blinded and placebo controlled design. Local, reversible, prolonged, and moderate pain and tenderness was reported both in patients and in controls after the combination compared to placebo. The pain was significantly more pronounced and tenderness tended also to be more pronounced in patients with ETTH compared to controls. Stimulus-response function, pressure pain detection thresholds (PPDT), total tenderness scores, cutaneous hyperalgesia, referred pain, and quality of pain after the combination indicated that peripheral sensitization of myofascial sensory afferents in ETTH was responsible for the hypersensitivity in these patients. However, central factors could not fully be excluded but play only a minor role. The baseline PPDT's were decreased in ETTH on the finger and in the temporal region compared to controls, which

might also be responsible for the mild hypersensitivity in patients after the combination.

In conclusion, a new experimental model of reversible, moderate and prolonged myofascial pain and tenderness using infusion of a combination of endogenous substances was developed. This model may be valuable in further investigations of the pathophysiological mechanisms behind myofascial pain disorders, their prevention and their treatment. Furthermore, patients with ETTH reported more pain and a trend to more tenderness after the combination compared to controls. It is hypothesized that peripherally increased excitability or sensitization of myofascial sensory afferents was responsible for this hypersensitivity in these patients.

Ingelise Christiansen

Vascular headaches with particular reference to the nitric oxide hypothesis and effect of modulation of the nitric oxide cascade

A PhD thesis based on the following works:
Christiansen I, Iversen HK, Olesen J, Tfelt-Hansen P. Nitric oxide-induced headache may arise from extracerebral arteries as judged from tolerance to isosorbide-5-mononitrate. *J Headache Pain* 2008; 9: 215–220.
Christiansen I, Daugaard D, Lykke Thomsen L, Olesen J. Glyceryl trinitrate induced headache in migraineurs – relation to attack frequency. *Eur J Neurol.* 2000; 7: 405–411.
Christiansen I, Iversen HK, Olesen J. Induction of nitrate tolerance is not a useful treatment in cluster headache. *Cephalalgia* 2000; 20: 445–454.
Christiansen I, Iversen HK, Olesen J. Headache characteristics during the development of tolerance to nitrates: pathophysiological implications. *Cephalalgia* 2000; 20: 437–444.
Christiansen I, Thomsen LL, Daugaard D, Ulrich V, Olesen J. Glyceryl trinitrate induces attacks of migraine without aura in sufferers of migraine with aura. *Cephalalgia* 1999; 19: 660–667; discussion 626.

Migraine and cluster headache have traditionally been classified as vascular headaches due to dilatation of cranial arteries during attacks. Studies, using the non-invasive ultrasound technique transcranial Doppler, point towards the intracranial arteries as the site of nociception. However, several pain-inducing mechanisms other than vasodilatation could be related to these headaches.

A decade of research into the molecular mechanisms of vascular headaches has evidenced that nitric oxide (NO) plays a key role. Thus, glyceryl trinitrate (GTN) (nitroglycerin), a prodrug of NO, causes migraine attacks in sufferers of migraine without aura, duster headache attacks in duster headache patients and non-specific vascular headaches in non-headache individuals. This thesis aimed at investigating the role of NO in migraine with aura, including whether an aura could be induced by GTN. Furthermore, the migraine-inducing ability of GTN was evaluated in relation to attack frequency by comparing the headache responses between sufferers of rare and frequent attacks of migraine without aura. Finally, it was analysed whether the induction of nitrate tolerance, using continuous administration of an organic nitrate, isosorbide-5-mononitrate (5-ISMN), could reduce attack frequency in duster headache patients and, thus, represent a novel therapeutic principle in vascular headaches. Insight into vascular headache mechanisms in general was aimed by correlating tolerance to headache with the adaptation of intra- and extra cerebral arteries in both healthy individuals and duster headache patients during long-term 5-ISMN administration. 12 sufferers of migraine with aura received intravenous infusion of GTN (0.5 μg/kg/min) for 20 min. 14 healthy subjects served as controls. Headache was more severe in migraineurs than in the controls during and immediately after GTN-infusion as well as during the following 11 hours. In the controls the GTN-induced headache gradually disappeared, whereas in migraineurs peak headache intensity occurred at a mean time of 240 minutes post-infusion. At this time the induced headache in 6 of 12 migraineurs fulfilled the diagnostic criteria for migraine without aura. The results therefore suggest that NO is involved in the pain mechanisms of migraine with aura. Since cortical spreading depression has been shown to liberate NO in animals, this finding may help our understanding of the coupling between cortical spreading depression and headache in migraine with aura.

In order to analyze whether the increased NO-sensitivity in migraineurs is related to the frequency of spontaneously occurring migraine attacks, intravenous infusion of *G'TN* (0.5 μg/kg/min for 20 min) was given to 15 migraine patients with rare attacks of migraine without aura. 14 age-matched migraine patients with frequent attacks of migraine without aura and 14 healthy subjects served as controls. No significant difference between migraine groups for any of several parameters was detected but both migraine groups experienced a headache that was significantly more severe, longer lasting and fulfilling the diagnostic criteria for migraine without aura more often compared to the healthy control group. This finding indicates that the increased sensitivity to GTN (NO) in migraineurs -also demonstrated in previous studies- is related to the status as a sufferer of migraine rather than to attack frequency, which probably relates to environmental influences.

Having established the key importance of NO in migraine, it seemed relevant to hypothesize that induction of nitrate (NO) tolerance (ie. an attenuation of the effects of nitrates (NO)) could be used therapeutically in vascular headaches. The knowledge that headache, a prominent adverse effect of nitrate therapy in patients with

cardiovascular diseases, often disappears during continuous treatment simultaneously with partly loss of vasodilatory effects, further supported this hypothesis. Furthermore, insight into tolerance to headache could lead to insight into vascular headache mechanisms in general. Therefore headache characteristics were recorded in 16 healthy subjects during 5-ISMN (30 mg t.i.d.) administration for 7 days in a double-blind, randomized placebo-controlled cross-over design until a state of tolerance to headache had developed. Blood velocity in the middle cerebral artery was measured with transcranial Doppler and the diameters of the temporal and radial arteries were measured with high frequency ultrasound. In lO subjects the headache fulfilled the *pain* subcriteria for migraine and in 5 subjects *all* diagnostic criteria for migraine without aura were fulfilled. Conversely, 20 min of intravenous infusion of GTN causes a milder headache and no migraine. The present results therefore suggest that NO may elicit a migraine attack in many/all healthy subjects if a high enough dose is given for several hours. A close temporal association between the disappearance of headache and the attenuation of the 5- ISMN induced dilatation of the superficial temporal artery was observed. In contrast, tolerance in the middle cerebral artery already appeared after 24 hours, which was earlier than the development of tolerance to headache. If vasodilatation is the cause of headache the results point to extra cerebral arteries. However, cytotoxic and pain modulating central nervous system effects of NO, the time course of which the appearance of tolerance is unknown, may also play a role, involving both intra and extra cerebral arteries.

In nine sufferers of chronic cluster headache 5-ISMN (30 mg t.i.d.) was administered orally for 4 weeks in a double-blind, randomized placebo-controlled cross-over design. Blood velocity in the middle cerebral artery was measured with transcranial Doppler and the diameters of the temporal and radial arteries were measured with high frequency ultrasound. The time profiles of both tolerance to nitrate-induced headache and tolerance of the cranial arteries were almost identical to the time profiles observed in healthy subjects but tolerance had no effect on cluster headache attack frequency. Conclusively, induction of tolerance to nitrates cannot be used to treat cluster headache and therefore probably migraine neither. The thesis nevertheless supports the important role of NO in vascular headaches and encourages the development of new treatments with the ability of reducing the formation of NO and for its effects.

Dorthe Daugaard

Involvement of the cerebral vascular system in spontaneous and nitric oxide-induced headache and migraine

A PhD thesis based on the following works:

Daugaard D, Thomsen LL, Olesen J. No relation between cephalic venous dilatation and pain in migraine. *J Neurol Neurosurg Psychiatry* 1998; 65: 260–262.

Daugaard D, Tfelt-Hansen P, Thomsen LL, Iversen HK, Olesen J. No effect of pure oxygen inhalation on headache induced by glyceryl trinitrate. *J Headache Pain* 2010; 11: 93–95

Daugaard D, Thomsen LL, Iversen HK, Olesen J. Delayed migraine-like headache in healthy volunteers after a combination of acetazolamide and glyceryl trinitrate. *Cephalalgia* 2009; 29: 1294–1300.

The purpose of the present thesis was to examine the involvement of parts of the cerebral vasculature in spontaneous and glyceryltrinitrate-induced headache. We examined the effect on headache during different conditions in both the venous and arterial cerebral system.

The cerebral vessels are surrounded by perivascular nerves that origin from the trigeminal nerve. Dilatation of the vessels may stretch these nerves and elicit pain. We examined the cerebral venous system during spontaneous migraine attacks, and induced a rapid increase in venous pressure with Queckenstedt's maneuver. We examined the effect on headache in the ongoing migraine attack. No significant worsening of the headache was found when compared to placebo, and this may argue against a major involvement of the cerebral venous system in spontaneous migraine.

The cerebral arterial system was examined using glyceryltrinitrate, which is a headache- and migraine inducing substance. Given glyceryltrinitrate healthy volunteers describe a mild to moderate, short-lasting headache, whereas migraineurs in addition, and often with a delay, suffer a delayed headache that fulfills the diagnostic criteria for migraine without aura. Glyceryltrinitrate liberates nitric oxide, which is a gas and diffuse across the vessel endothelium and induces dilatation of the vessels by relaxing vascular smooth muscles. Nitric oxide is believed to be involved in the disease mechanism of migraine. A concurrent vasodilation in the middle cerebral arteries, using Transcranial Doppler, has been reported during the immediate headache following infusion of glyceryltrinitate in both healthy volunteers and migraine patients. We induced a migraine attack in eighty percent (16 out of 20) with known spontaneous migraine without aura, with glyceryltrinitrate. The mean blood velocity in the middle cerebral arteries decreased rapidly during infusion, and increased slowly without reaching baseline at the end of the study. A correlation between the headache intensity and the mean blood velocity decrease could not be found.

Nitric oxide's half-life is seconds, and it reacts with oxygen and is decomposed to mainly NO_x-compounds. Healthy volunteers were given oxygen inhalations prior to a Glyceryltrinitrate-infusion in order to increase the

breakdown of nitric oxide and thereby reduce headache. We found no decrease in headache following oxygen compared to placebo, although a trend points towards lesser headache intensity following oxygen. Oxygen is crucial in many biological reactions, and the amount of oxygen may have been insufficient.

Acetazolamide predominantly dilates the intracranial arterioles, and glyceryltrinitrate almost selectively dilates the arteries. Relieving the increased pressure in the arteries following glyceryltrinitrate and thereby reduce the stretching of the arterial vessel walls may theoretically reduce the headache intensity. We pretreated healthy volunteers with acetazolamide, before administering a GTN-infusion, and examined the effect on the induced headache. The immediate headache following acetazolamide and glyceryltrinitrate did not differ from placebo and glyceryltrinitrate. The participants experienced a delayed headache hours after acetazolamide and glyceryltrinitrate, and three subjects fulfilled the criteria for migraine without aura. The mean blood velocity increased by 38% in 30 minutes after acetazolamide administration, which is comparable to other studies. Glyceryltrinitrate induced an identical, immediate 20% decrease in mean blood velocity on both days. We suggest that the delayed headache may originate from release of nitric oxide secondary to shear stress. Future research may determine the detailed involvement of the cerebral arterial system in migraine, in which the glyceryltrinitrate-model of experimental headache has proven to be a very useful tool.

Lise Lykke Thomsen

An epidemiological and clinical investigation of hemiplegic migraine

A PhD thesis based on the following works:
Lykke Thomsen L, Kirchmann Eriksen M, Faerch Romer S, Andersen I, Ostergaard E, Keiding N, Olesen J, Russell MB. An epidemiological survey of hemiplegic migraine. *Cephalalgia* 2002; 22(5): 361–375.
Thomsen LL, Eriksen MK, Rømer SF, Andersen I, Olesen J, Russell MB. A population-based study of familial hemiplegic migraine suggests revised diagnostic criteria. *Brain* 2002; 125(Pt 6): 1379–1391.
Thomsen LL, Østergaard E, Rømer SF, Andersen I, Eriksen MK, Olesen J, Russell MB. Evidence for a separate type of migraine with aura: sporadic hemiplegic migraine. *Neurology* 2003; 60: 595–601.

The objective of this PhD thesis was to use a systematic population based approach in order to estimate the prevalence of hemiplegic migraine (HM), to describe the clinical characteristics of HM and to compare HM to previous described characteristics of migraine with typical aura (MA). HM has been described in a familial (FHM) and a sporadic (SHM) form. Both forms are rare and probably therefore systematic epidemiological studies have never been carried out. The diagnostic criteria of the International Headache Society were used in a systematic search employing 3 different search strategies to the entire Danish population: The National Patient Register, case records and advertisements. First 1446 recruited patients were screened. Secondly all affected and their 859 relatives were screened in an extensive validated semi structured telephone interview. Identified affected HM patients had a face to face interview and a neurological examination. The prevalence was estimated by means of the statistical method known as capture-recapture.

Of the 291 identified patients, 147 were FHM from 44 different families, 105 were SHM and 39 were unclassifiable. The prevalence of HM was estimated to be 0.01%. The clinical characteristics of FHM and SHM were almost identical but different from MA. Based on these data and existing genetic data, we suggest more precise diagnostic criteria for FHM and separate diagnostic criteria for SHM. Furthermore, our data suggest differences in the pathophysiological mechanisms of HM and MA that indicate a different genetic background. The patients presented in this thesis offer an excellent opportunity to test this hypothesis and furthermore to identify new genes for FHM in future studies.

Messoud Ashina

Neurobiology of chronic tension-type headache

A doctoral thesis in medical science based on the following works:
Ashina M, Lassen LH, Bendtsen L, Jensen R, Olesen J. Effect of inhibition of nitric oxide synthase on chronic tension-type headache: a randomised crossover trial. *Lancet* 1999; 353: 287–289.
Ashina M, Bendtsen L, Lassen LH, Jensen R, Sakai F, Olesen J. Possible mechanisms of action of nitric oxide synthase inhibitors in chronic tension-type headache. *Brain* 1999; 122:1629–1635.
Ashina M, Bendtsen L, Jensen R, Ekman R, Olesen J. Plasma levels of substance P, neuropeptide Y and vasoactive intestinal polypeptide in patients with chronic tension-type headache. *Pain* 1999; 83: 541–547.
Ashina M, Bendtsen L, Jensen R, Olesen J. Nitric oxide-induced headache in patients with chronic tension-type headache. *Brain* 2000; 123:1830–1837.
Ashina M, Bendtsen L, Jensen R, Jansen-Olesen I, Schifter S, Olesen J. Plasma levels of calcitonin gene-related peptide

in chronic tension-type headache. *Neurology* 2000; 55:1335–1339.
Ashina M, Bendtsen L, Jensen R, Schifter S, Olesen J. CGRP levels during nitric oxide-induced headache in patients with chronic tension-type headache. *Eur J Neurol* 2001; 8: 173–178.
Ashina M, Stallknecht B, Bendtsen L, Pedersen JF, Galbo H, Olesen J. *In vivo* evidence of altered skeletal muscle blood flow in chronic tension-type headache. *Brain* 2002; 125: 320–326.

The purpose of the present thesis was to study the neurobiology of chronic tension-type headache. It has been hypothesized that sensitization of second order neurons may play an important role in the pathophysiology of chronic tension-type headache. Nitric oxide (NO) appears to play an important part in the process of central sensitization. To test the hypothesis NO mechanisms in chronic tension-type headache were investigated in four studies. In the first study, the analgesic effect of the nitric oxide synthase (NOS) inhibitor, L-N^G methyl arginine hydrochloride (L-NMMA) was investigated in patients with chronic tension-type headache. L-NMMA reduced headache intensity significantly more than placebo. To explore the mechanisms of this antinociceptive effect myofascial factors in relation to NOS-inhibition were studied in the second study. Both muscle hardness and tenderness were significantly reduced following treatment with L-NMMA, while there was no significant reduction at any time after treatment with placebo. It was concluded that the antinociceptive effect of NOS inhibition in patients is most likely due to reduction of central sensitization at the level of the spinal dorsal horn/trigeminal nucleus{Ashina, Lassen, et al., 1999, 200/id}{Bendtsen, 2000, 1099/id}. In the third study, the NO donor glyceryl trinitrate (GTN) model of experimental headache was used to study whether NO may induce or enhance central sensitization in chronic tension-type headache. GTN infusion in patients resulted in biphasic response with an immediate and a delayed headache. Patients developed significantly stronger immediate and delayed headache after intravenous infusion of GTN than after infusion of placebo. Furthermore, patients developed significantly stronger headache after GTN than healthy controls. In the fourth study, mechanisms of the immediate headache were investigated by measuring plasma concentration of calcitonin gene-related peptide (CGRP) before, during and after infusion of GTN and placebo. No significant changes in plasma CGRP after GTN infusion were found in either patients or controls. This indicates that immediate headache after GTN infusion in patients is not mediated by release of CGRP. It was concluded that it is most likely that immediate headache after infusion of GTN originates from direct action of NO on perivascular sensory nerves or from NO-induced arterial dilatation, or both, while the delayed headache may be due to augmentation of pre-existing central sensitization. To explore possible role of neuropeptides in chronic tension-type headache, plasma levels of CGRP and substance P (SP), neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) were measured in the cranial and peripheral circulation of patients and controls. The results indicate that plasma levels of CGRP, SP, NPY and VIP are normal in patients and largely unrelated to headache state. However, exploratory testing in relation to headache characteristics showed that patients with a pulsating pain quality had higher plasma CGRP in the headache free period than controls. It was suggested that these patients might pathophysiologically be related to migraine. Finally, a microdialysis technique was applied to study muscle blood flow in tender muscle of patients with chronic tension-type headache during static work and compared to muscle blood flow in non-tender muscle of healthy subjects. Patients had significantly lower increase in blood flow in response to static exercise than controls. It was concluded that the central neuroplastic changes might affect the regulation of peripheral mechanisms and thereby lead to increased tenderness and chronic headache. Overall, the present thesis contributes to understanding of the complex mechanisms leading to chronic tension-type headache and provides data that will hopefully lead to new treatment modalities.

Steffen Birk

On cAMP signaling in cerebral haemodynamics-implications for migraine pathophysiology

A PhD thesis based on the following works:
Birk S, Edvinsson L, Olesen J, Kruuse C. Analysis of the effects of phosphodiesterase type 3 and 4 inhibitors in cerebral arteries. *Eur J Pharm* 2004; 489: 93–100.
Birk S, Petersen KA, Kruuse C, Guieu R, Jonassen O, Eisert W, Olesen J. The effect of circulating adenosine on cerebral hemodynamics and headache. *Cephalalgia* 2005; 25: 369–377.
Birk S, Kruuse C, Petersen KA, Jonassen O, Tfelt-Hansen P, Olesen J. The phosphodiesterase 3 inhibitor cilostazol potently dilates large cerebral arteries in man without affecting cerebral blood flow. *J Cereb Blood Flow Metab* 2004; 24: 1352–1358.

The present thesis concerns aspects of the role of cyclic adenosine monophosphate (cAMP) in the pathogenesis of migraine using our human vascular headache model. Migraine is a prevalent disorder with considerable impact on the individual migraine patient and socioeconomics. Nitric oxide (NO) has previously been suggested to play a key role in migraine pathogenesis, possibly by dilating cerebral arteries and/or sensitising perivascular afferents in the trigeminovascular system. On the molecular level,

the headache-generating effect of NO is mediated by cyclic guanosine monophosphate (cGMP). However, the endogenous signal substances adenosine and calcitonin gene-related peptide are proposed to play a role in rnigraine and the effect of these substances is believed to be mediated by cAMP. We therefore decided to investigate the effect of cAMP on headache generation and to investigate possible mechanisms involved such as cerebral artery dilatation and sensory changes.

For research tools we used adenosine, whose vasodilator and pro-nociceptive actions are mediated by increases in cAMP; and cilostazol, an anti-platelet and vasodilator drug that attenuates cAMP hydrolysis by inhibiting phosphodiesteras e type 3 (PDE3). These substances were administered to healthy volunteers with no personal or family history of migraine in a randomised, double-blind, erossover design. Headache response was studied with a verbal rating scale and headache characteristics were recorded according to the International Headache Society (IHS). Regional cerebral blood flow (rCBF) using ^{133}Xe inhalation and single photon emission computerized tomography (SPECT) and middle cerebral artery blood flow velocity Vmca) measured with transcranial Doppler. Obtaining rCBF and Vmca values in direct sequence allowed assessment of MCA diameter changes. Radial and temporal artery diameter was measured with high-resolution ultrasonograph y and mechanical pain thresholds were measured in the region innervated by the lst division of the trigeminal nerve and in a peripheral control site (cilostazol study only).

In addition to the clinical studies, in vitro pharmacology was used to assess the effect of PDE3 and PDE4 inhibition in isolated cerebral arteries and to investigate the cyclic nucleotides involved in the relaxant response to cilostazol and reference PDE inhibitors.

The in vitro pharmacological studies confirmed that PDE3 plays a major role in cAMP hydrolysis in both human and guinea pig cerebral arteries. Functionally, cilostazol dilated pre contracted guinea pig basilar arteries with a concomitant increase in cAMP. Due to methodological limitations a clear relationship between cAMP and relaxant response could, however, not be established. Cilostazol and a highly selective PDE3 inhibitor cilostamide, also attenuated cGMP hydrolysis, but cGMP did not appear to play a role in the relaxant response to cilostazol in clinically relevant doses ($<$ 10 11M).

Cilostazol 200 mg p.o. induced headache in 11 of 12 volunteers compared to 2 after placebo with a mean peak: 6–9 hours post-dose, which was 2–5 hours after the last data calleetion point. The pain quality associated with the headache often had migraine-like features and 2 of completed vblunteers developed attacks fulfilling IHS criteria for migraine without aura. Cilostazol significantly increased MCA, superficial temporal and radial artery diameter at least to a similar extent as previously tested headache-generating vasodilators GTN and CGRP. No changes in mechanical pain thresholds could be detected.

Adenosine was tested using 80 f. Lgrng- 1!ili.in-1 and 120!lgrng-111hin-1 vs. placebo. There was an overall significant increase in headache score between treatments, but only 2 volunteers reported actual head painduring either adenosine. The maximal response observed was 2 on the O-l O scale. The remaining volunteers reported an altered, pressing or throbbing sensation, which may or may not be a sub-clinical type of pain. Six volunteers developed light to moderate delayed headache 3–8*li* h post-dose. Adenosine caused marked hyperventilation, but did not itself affect CBF or VMeA, thus MCA diameter remained unchanged. Themost likely explanation is that adenosine was degraded in the vascular endothelium before reaching receptors in vascular smooth muscle cells or periva scular afferents. Adenosine was therefore not a useful tool in investigating cAMP mechanisms in migraine and found littie evidence for *circulating* adenosine playing a role in spontaneous migraine attacks as previously proposed.

These tindings support a role of cAMP in migraine pathogenesis. The mechanism involved may be dilatation of large cerebral arteries, but recent studies question this hypothesis. Peripheral or central sensitisation of trigemirral afferents has been suggested as a possible mechanism. We found no results supporting this, but the study was not primarily designed to investigate this in detail. Further investigations of the role of cAMP in migraine and the molecular and functional downstream mechanisms involved seem warranted, as does the investigation of the level at which cGMP and cAMP signalling interact or converge in relation to cerebrovascular dilatation and migraine pathogenesis.

Jesper Filtenborg Tvedskov

A human experimental migraine model using glyceryl trinitrate for evaluating prophylactic treatment

A PhD thesis based on the following works:

JF Tvedskov, LL Thomsen, HK Iversen, A Gibson, P Williams, J Olesen. The prophylactic effect of valproate on glyceryl trinitrate induced migraine. *Cephalalgia* 2004; 24: 576–585.

JF Tvedskov, LL Thomsen, LL Thomsen, HK Iversen, P Williams, A Gibson, K Jenkins, R Peck and J Olesen. The effect of propranolol on glyceryl trinitrate induced headache and arterial response. *Cephalalgia* 2004; 24: 1076–1087.

JF Tvedskov, HK Iversen, J Olesen. A double blind study of SB-220453 (Tonerbasat) in the Glyceryl trinitrate (GTN) model of migraine. *Cephalalgia* 2004; 24: 875–882.

This PhD thesis is based on three studies and evaluates the glyceryl trinitrate (GTN) migraine model and broaden its use to test new antimigraine drugs.

The nitric oxide donor glyceryl trinitrate (GTN), besides inducing immediate headache during infusion, induces delayed migraine attacks in patients suffering from migraine that are indistinguishable from their spontaneous attacks. The GTN model of migraine was modified for the testing of prophylactic drugs. Two validation studies investigated the use of the model with established prophylactic migraine drugs. In the third study, the model was used in the testing of a new potentially antimigraine drug.

In the first study twelve patients with migraine without aura were included in a randomized double blind crossover study. Valproate or placebo were each given daily for a minimum of 13 days. On the last treatment day, a 20 min intravenous infusion of GTN 0.25 μg/min was given. Headache intensity was registered for 12 hours and fulfilment of the diagnostic criteria for migraine without aura of the International Headache Society (IHS migraine) was recorded for 24 hours. The GTN induced headache and migraine were reduced after valproate compared to placebo, although only one parameter was statistically significantly reduced. Pre-treatment with valproate, as compared to placebo, reduced blood flow velocity in both middle cerebral arteries (MCA) after GTN. No effect of valproate was seen in the diameter of the superficial temporal or the radial arteries before or after GTN. The study indicates that the prophylactic effect of valproate can be demonstrated using the GTN migraine model.

In the second study propranolol was used in the same set-up. Fourteen migraine patients and 14 sex and aged matched healthy subjects completed the study. All migraine subjects and healthy subjects developed headache after GTN. No effect of propranolol on GTN induced headache and migraine was found. Headache after GTN was more pronounced in migraine subjects than in healthy subjects both with and without pre-treatment of propranolol. Two weeks of propranolol constricted the radial artery in healthy subjects, but not in migraine patients and the GTN induced vasodilatation abolished that difference. The MCA blood flow velocity was higher in healthy subjects than in the migraine patients and it remained unaffected by propranolol. These findings indicate that GTN induces migraine at a deeper level of the pathophysiological cascade of migraine than the prophylactic effect of propranolol. Propranolol does not constrict cerebral arteries, which therefore cannot be part of its mechanism of action in migraine.

In the third study a new potentially antimigraine drug, SB-220453 (Tonerbasat), was tested in the GTN model of migraine. In this randomized double blind crossover study 15 migraine patients were pre-treated with SB-220453 40 mg or placebo. The GTN infusion of 0.5 μg/kg/min was consistent in producing headache and migraine that resembled the patients' usual spontaneous migraine.

However, the study was terminated prematurely due to interaction between SB-220453 and GTN resulting in hypotensive episodes. Yet, the peak headache score showed a trend towards reduction after SB-220453 compared to placebo, but only 9 patients completed the study and that did not allow any conclusion other than that SB-220453 had no major preventive antimigraine activity compared to placebo in the GTN model.

The GTN model is a safe and reliable method of inducing headache and migraine, and it may be of value for testing new prophylactic antimigraine drugs. Both the GTN dose and the endpoints should be chosen carefully and also the u se of rescue medication can create endpoint difficulties. Therefore, studies should be carried out in a centre that is familiar with the model.

Kenneth Ahrend Petersen

CGRP and CGRP-antagonism – An investigation of vascular headache pathophysiology and treatment

A PhD thesis based on the following works:
Petersen KA, Dyrby L, Williamson D, Edvinsson L, Olesen J. Effect of hypotension and carbon dioxide changes in an improved genuine closed cranial window rat model. *Cephalalgia* 2005; 25: 23–30
Petersen KA, Birk S, Lassen LH, Kruuse C, Jonassen O, Lesko L, Olesen J. The CGRP antagonist BIBN4096BS does not affect cerebral or systemic haemodynamics in healthy volunteers. *Cephalalgia* 2005; 25: 139–147
Petersen KA, Birk S, Doods H, Edvinsson L, Olesen J. Inhibitory effect of BIBN4096BS on cephalic vasodilatation induced by CGRP or transcranial electrical stimulation in the rat. *British J Pharmacol* 2004; 143: 697–704

The present PhD-thesis aimed to introduce an improved animal model of vascular headache to the field of migraine research. By application of this model and a previously validated human experimental model of migraine, the role of CGRP in the pathogenesis of vascular headache and the possible use of CGRP receptor antagonism in the treatment of headache with a vascular genesis was further investigated.

CGRP is a potent dilator of cerebral and extra cerebral arteries and vasodilatation is believed to play an important role in the pathophysiology of vascular headache. CGRP is located in perivascular sensory nerve fibers and its plasma level is increased during attacks of migraine and duster headache. A previous study showed that after treatment of a migraine attack with sumatriptan or upon spontaneous cessation of the attack, plasma levels of CGRP was normalized. In 8 out of 9 migraine patients an immediate

migraine-like headache was induced by i.v. CGRP and approximately 5.5 hour later all patients experienced a delayed headache, fulfilling the criteria for migraine without aura in approximately 30% of the patients. Another study to support involvement of CGRP in migraine pathogenesis demonstrated that the CGRP receptor antagonist BIBN4096BS was effective in the acute treatment of migraine 7.

Initially, an existing animal migraine model was improved by adding measurements of pial arteries (PA) and local cerebral blood flow by laser Doppler flowmetry (LCBFFJux). By modifying the animal model, a closer resemblance to established experimental human migraine model was obtained. Our results from the validation of the model emphasized that $PaCO_2$ must be tightly controlled and that effect of blood pressure changes must be known to interpret pharmacological experiments in this model.

The improved animal model was used to investigate the inhibitory properties of BIBN4096BS and assess where the anti-migraine effect of the compound took place. BIBN4096BS inhibited the CGRP induced decrease in mean arterial blood pressure and the increase of MMA diameter. It could, however, not significantly inhibit CGRP induced increase in PA diameter and LCBFFlux· Presynaptic CGRP release due to transcranial electrical stimulation was not inhibited. The tentative conclusion was that BIBN4096BS does not readily cross the blood-brain barrier and BIBN4096BS most likely exerts its anti-migraine action on dural or other extracerebral vessels.

Subsequently, we performed a study in healthy volunteers using the validated human experimental model and examined whether BIBN4096BS blockade of the receptor would result in vasoconstriction. This did not seem to be the case, since even a dose 4 times higher (10 mg) than the minimal effective dose (2.5 mg) did not have any effect on measured CBF, mean blood flow velocity in the middle cerebral artery, temporal and radial arterial diameter, or systemic hemodynamics.

Ann Lyngberg

Migraine and tension-type headache: Prevalence incidence prognosis and impact

A PhD thesis based on the following works:

Lyngberg AC, Rasmussen BK, Jørgensen T, Jensen R. Has the prevalence of migraine and tension-type headache changed over a 12-year period? A Danish population survey. *Eur J Epidemiol* 2005; 20: 243–249.

Lyngberg AC, Rasmussen BK, Jørgensen T, Jensen R. Incidence of primary headache. A Danish epidemiological follow-up study. *American Journal of Epidemiology* 2005; 161(11): 1066–1073.

Lyngberg AC, Rasmussen BK, Jørgensen T, Jensen R. Prognosis of migraine and tension-type headache: A population-based follow-up study. *Neurology* 2005; 65(4): 580–585.

Lyngberg AC, Rasmussen BK, Jørgensen T, Jensen R. Secular changes in health care utilization and work absence for migraine and tension-type headache. A population based study. *Eur J Epidemiol* 2005; 20(12): 1007–1014

The present PhD thesis was carried out at the Danish Headache Center and the Research Centre for Prevention and Health in Glostrup. The aims of the PhD study were to estimate the incidence and prognosis of migraine and tension-type headache in the general population and to identify possible determinants. Moreover, the aims were to assess changes in migraine and tension-type headache prevalence, consultation rates, medication use, and work absences over a 12-year period.

The PhD study comprises a cross-sectional survey of 300 subjects aged 25–36 years and a follow-up survey of 1000 subjects aged 37–76 years from a cross-sectional population-based survey in 1989. Data collection was conducted between May 2001 and April 2002. All eligible subjects were invited to a medical interview and a general examination with emphasis on primary headaches. Non-responders were asked to complete the medical interview by telephone. Migraine and tension-type headache diagnoses were assessed by a medical doctor and based on ICHD-1 and ICHD-11. In total, 848 (72%) of 1175 eligible subjects participated, hereof 555 in face-to-face interviews and 293 in telephone interviews. In the follow-up part, 549 (82%) of 673 eligible participated. The participants were representative of the eligible study populations with regard to gender and age.

The one-year prevalence of migraine (11% to 15%, $p = 0.21$) was stable from 1989 to 2001 but the proportion of migraineurs with more than 14 migraine days per year increased from 12% to 38% ($p = 0.03$). The overall one-year prevalence of tension-type headache (79% to 87% $p = 0.04$) and the prevalence of frequent episodic tension-type headache (29% to 37%, $p = 0.03$) increased, while the prevalence of chronic tension-ty pe headache tended to increase (1.8% to 4.8%, $p = 0.08$). The majority of migraineurs (92–94%) also reported tension-type headache, and 40% reported coexisting frequent episodic or chronic tension-type headache.

The incidence of migraine was 8.1 per 1000 with a male: female ratio of about 1:6. Besides being young or female, risk factors were familial disposition, no vocational education, high work load and frequent tension-type headache. For frequent episodic or chronic tension-type headache, the incidence was 14.2 per 1000. The male:female ratio was about 1:3. Risk factors were young age, female gender, poor self-rated health, inability to relax after work, and sleeping fewer hours per night.

Poor outcome (>14 migraine days per year) was observed in 20% of 64 migraineurs: 13% of migraineurs with low frequency and 60% of migraineurs with high frequency at baseline. Besides high migraine frequency at baseline, age at onset below 20 years was a risk factor. Of 161 subjects with frequent episodic or chronic tension-type headache at baseline, poor outcome (chronic tension-type headache) was experienced by 16%. 12% of subjects with frequent episodic tension-type headache and 53% of subjects with chronic tension-type headache had poor outcome. Besides baseline chronic tension-type headache, predictive factors were coexisting migraine, not being married and sleeping problems.

Headache-related consultation rates, especially consultations with specialist, increased from 1989 to 2001, while overall consultation rates and overall and headache-related absence rates were stable. Use of headache-related prescribed medication increased moderately, whereas the use of headache prophylactics was stable at a low rate. Consultations and absence rates were higher for subjects with headache than for healthy subjects and highest for subjects with both migraine and tension-type headache. Migraine was associated with high headache-related absence rates, while frequent tension-type headache was associated with high overall absence rates. Among migraineurs, triptan users had higher migraine headache frequency and tended to have higher absence rates than non-users.

In conclusion, the increases in frequency and health care utilisation indicate higher impact of migraine and tension-type headache in 2001 than in 1989. The highest impact was seen for coexisting migraine and tension-type headache, which illustrates the need for assessing all headache diagnoses independently. Furthermore, the findings emphasize the need for disentangling the interaction of headache frequency, comorbidities, consultations, and medication use for migraine and tension-type headache. The observed risk factors may yield novel approaches to preventive or interventional strategies and suggest further exploration of the possibly genetic background of high frequency migraine, the link between stress and migraine, and the role of sleep and comorbidities such as depression and anxiety in tension-type headache. Longitudinal or replicate studies of primary headaches in the general population are few and tension-type headache or coexisting migraine and tension-type headache are seldom addressed. The present study is small in scale, but, due to the high quality of data obtained by clinical interviews, the results may possibly constitute a reference material for future headache research.

Malene Kirchmann

Migraine with aura – New understanding of diagnosis and classification from clinical epidemiological studies

A PhD thesis based on the following works:
Eriksen MK, Thomsen LL, Andersen I, Nazim F, Olesen J. Clinical characteristics of 362 patients with familial migraine with aura. *Cephalalgia* 2004; 24: 564–575.
Eriksen MK, Thomsen LL, Olesen J. Sensitivity and specificity of new diagnostic criteria for migraine with aura. *J Neurology Neurosurg Psychiatry* 2005; 76: 212–217.
Eriksen MK, Thomsen LL, Olesen J. New international classification ofmigraine with aura (ICHD-2) applied to 362 migraine patients. *Eur J Neurol* 2004; 11: 583–591.
Eriksen MK, Thomsen LL, Olesen J. The Visual Aura Rating Scale for migraine aura diagnosis. *Cephalalgia* 2005: 25: 801–810.

Migraine with typical aura (MTA) is characterized by attacks of reversible visual, sensory or aphasic aura symptoms. The diagnosis of MTA and the documentation of subtypes of MTA depend on the description of symptoms because there are no diagnostic biological markers available though epidemiological studies suggest an underlying genetic susceptibility to MTA. The aims of the present thesis were to develop new operational diagnostic criteria for MTA for the second edition of the International Classification of Headache Disorders (ICHD-2); to develop a Visual Aura Rating Scale (VARS) as a supplementary tool for diagnosing MTA; to describe the clinical characteristics of familial MTA; and to identify clinical subtypes of MTA based on family history and phenotype variation.

The studies were based on the analysis of the clinical characteristics of MTA. The participants were recruited from specialist practice and diagnosed in a validated semi-structured physician-conducted telephone interview. The participants comprised 362 patients with MTA according to the first edition of the International Classification of Headache Disorders (ICHD-1) plus 112 patients with reversible non-aura visual disturbances not fulfilling the ICHD-1 for MTA. The diagnosis of the patients with MTA was supported by a long history of MTA, a history of previous migraine diagnosis and treatment and a familial predisposition to MTA.

For the development of the ICHD-2 for MTA the 474 participants were separated into a derivation sample and a validation sample. Selected sets of criteria were tested for sensitivity and specificity comparing with the diagnosis according to the ICHD-1. The set of criteria selected for the ICHD-2 had a sensitivity of 85% and a specificity of 97% in the derivation sample, and a sensitivity of 90% and a specificity of 96% in the validation sample. According to this set of criteria the patient has to fulfil at least two of the following: 1) homonymous visual symptoms and/or unilateral sensory symptoms. 2) at least one aura symptom develops gradually over at least 5 min and/or different symptoms occur in succession over 25 min. 3) each symptom lasts between 5 and 60 min. According to the ICHD-2,

MTA is further sub-classified according to the characteristics of the headache following the aura.

For the development of VARS the participants were subsequently diagnosed according to the ICHD-2 and separated into a derivation sample and a validation sample. By regression analysis we identified the visual aura characteristics associated with MTA in the derivation sample. Based on the identified characteristics we developed VARS and derived a predictive VARS score which was tested in the validation sample. The VARS score is the weighted sum of the presence of five visual symptom characteristics: duration 5–60 min. (3 points), develops gradually over at least5 min. (2 points), scotoma (2 points), zig-zag lines (2 points), and unilateral (1 point). The maximum score is IO points. A VARS score of 5 or more diagnosed MTA with a sensitivity of 96% and a specificity of 98% in the derivation sample, and a sensitivity of 91% and a specificity of 96% in the validation sample.

Subgroups of patients with MTA showed clinical differences. Among patients with a strong familial predisposition to MTA the age at onset of MTA was lower (15 ± 6 vs. 21 ± 12 years, $p = 0.001$) and the age at cessation of attacks of MTA tended to be higher than in patients with few affected relatives. Patients with familial MTA were more likely to experience two or more aura symptoms than patients with population-based MTA (61% vs. 32%, $p < 0.0001$). Within the ICHD-2 subtypes of MTA, patients with typical aura with migraine headache had an earlier age at onset (20 ± 10 vs. 23 ± 13 years $p = 0.044$) and a higher co-occurrence of migraine without aura (43% vs. 22%, $p = 0.002$) than patients with typical aura with non-migraine headache.

VARS and the ICHD-2 for MTA are more operational and probably delineate a more homogeneous sample of patients with MTA than the ICHD-1. Eventually, the validity of VARS and the ICHD-2 for MTA has to be tested against biological markers such as the genetic constitution of MTA. The families with MTA from the present study will be used for a genome wide scan to identify susceptibility genes in MTA. The identification of clinical subtypes of MTA may serve as an approach for stratifying patients for these genetic studies as MTA is highly likely a genetically heterogeneous disorder.

Aydin Gozalov

Role of K+ channels in cephalic vasodilation- implications for migraine pathophysiology

A PhD thesis based on the following works:
Gozalov A, Petersen KA, Mortensen C, Jansen-Olesen I, Klaerke, Olesen J. Role of KATP channels in the regulation of rat dura and pia artery diameter; *Cephalalgia* 2005; 25: 249–260.
Gozalov A, Jansen-Olesen I, Klaerke D, Olesen J. Role of KATP channels in cephalic vasodilatation induced by CGRP, NO and transcranial electrical stimulation in the rat. *Headache* 2008; 48: 1202–1213.
Gozalov A, Jansen-Olesen I, Klaerke D, Olesen J. Role of BKca channels in cephalic vasodilatation induced by CGRP, NO and transeramal electrical stimulation in the rat. *Cephalalgia.* 2007; 27:1120–1127.

The purpose of the present thesis was to study the role of K+ channels in migraine pathogenesis and their involvement in CGRP and NO induced headache. Both CGRP and NO are potent vasodilators that can induce migraine and their antagonists are effective in the treatment of migraine attacks.

The discovery of ion channel dysfunction in the pathogenesis of migraine makes K+ channels a relevant target in headache research. Moreover, most of the patients experienced headache when KATP channels agonists are used for asthma. In the thesis, focus is directed towards the effect of KATP and BKca channels on NO- and CGRP-induced cranial vasodilatation, because these molecules play a causal role in migraine. All studies were based on an improved genuine closed cranial window model, supported by *in vitro* experiments.

The main findings of the present study were:

That KATP channel openers preferentially dilate dural artery, a mechanism thought to be involved in migraine pathogenesis and possibly in the headache generating effect of KATP channel openers.

That the KATP channel selective blocker glibenelamide can attenuate CGRP- but not GTN induced dural artery dilatation *in vivo,* suggesting a role of KATP channels in CGRP-induced meningeal vasodilatation in the rat.

In the rat, activation of BKca but not KATP channels has been observed to contribute to

GTN/NO-induced vasodilatation both *in vivo* and *in vitro,* suggesting a role of BKca channels in NO-induced vasodilatation in the rat

Thus, it seems that NO and CGRP in rat dural and pial circulation act via two different types of K+ channels. Our data suggest that interaction of KATP and BKca channels is implicated in the development of vascular headaches.

Lars Schack Kruse

Molecular mechanisms involving cyclic nucleotides in migraine

A PhD thesis based on the following works:
Kruse LS, Sandholdt NTH, Gammeltoft S, Olesen J, Kruuse C. Phosphodiesterase 3 and 5 and cyclic nucleotide-gated ion channel expression in rat trigeminovascular system. *Neurosci Lett.* 2006;404: 202–207

Kruse LS, Tibæk M, Gammeltoft S, Møller M, Olesen J, Kruuse C. PDE9A, PDE10A, and PDE11A expression in rat trigeminovascular pain signalling system. *Brain Res.* 2009; 1281: 25–34
Schankin C, Kruse LS, Reinisch V, Jungmann S, Kristensen JC, Grau S, Ferrari U, Sinicina I, Goldbrunner R, Straube A, Kruuse C. Nitric oxide induced changes in endothelial expression of phosphodiesterases 2, 3 and 5 *Headache;* 50: 431–441.

Migraine is a disease of unknown aetiology. Several theories have been developed over the years, but consensus is presently moving towards a neurovascular model in which interplay between the large cerebral arteries and the nociceptive perivascular neurons are thought to be involved in the initial triggering mechanism. It is well-established that the mechanism involves activation of afferent fibres of the trigeminovascular system. Numerous reports have indicated a role for elements of cyclic nucleotide signaling in the disease process – e.g. nitric oxide, calcitonin gene-related peptide, ion channels and more recently phosphodiesterases, but where and how is uncertain.

In this thesis the main focus is on characterisation of some of the down-stream effectors of cyclic nucleotide signalling in tissues related to migraine. Although these also include protein kinases and Epacs we have chosen to focus on the cyclic nucleotide-gated ion channels and the phosphodiesterases. We find a widespread, although clearly non-uniform expression of both groups of proteins in tissues of the trigeminovascular system. Given the fact that the PDE3 and PDE5 inhibitors, Cilostazol and Sildenafil, cause headache and migraine these findings may further strengthen the hypothesis of PDEs as drug targets in migraine. A role for cyclic nucleotide-gated channels is less clear. We also examined if expression of a selection of PDEs would be regulated compensatorily under sustained NO-stimulation by the long-lasting donor DETA-NONOate in a cell-based assay. Although the mRNA data suggested that indeed the PDEs were regulated according to the typical bi-phasic time course of a migraine attack, the protein analysis did not significantly back these observations. In conclusion, there is improved evidence for the expression of a variety of PDEs in the trigeminovascular system and this may implicate them in the modulation of the pain pathway in migraine. Given that most migraine-inducing substances, or proteins whose mutation is linked to migraine, work through modulation of production of cyclic nucleotides it may be that PDE-selective targeting in future migraine drug regimes may be a more efficient and pharmacologically safer approach.

Line Buchgreitz

Pain processing in tension-type headache

A PhD thesis based on the following works:
Buchgreitz L, Lyngberg AC, Bendtsen L, Jensen R. Frequency of Headache is related to sensitization: a population study. *Pain* 2006; 123: 19–27.
Buchgreitz L, Lyngberg A, Bendtsen L, Jensen R. Increased prevalence of tension-type headache over a 12-year period is related to increased pain sensitivity. A population study. *Cephalalgia* 2006; 27: 145–152.
Buchgreitz L, Lyngberg AC, Bendtsen L, Jensen R. Increased pain sensitivity is not a risk factor but a consequence of frequent headache: A population-based follow-up study. *Pain* 2008; 137: 623–630.
Buchgreitz L, Egsgaard LL, Jensen R, Arendt-Nielsen L, Bendtsen L. Abnormal pain processing in tension-type headache: A high-density EEG brain mapping study. *Brain* 2008; 131: 3232–3238.

Based on growing evidence from pain perception studies, it has been hypothesized that frequent nociceptive input from muscles in the cephalic region induces central sensitization. This impaired supraspinal modulation in the central nervous system leads to chronic tension-type headache and generalized hyperalgesia. This hypothesis is mainly based on studies performed on severely affected patients from headache clinics. It is important to examine whether the knowledge obtained in these highly selected patients is valid in the general population. Moreover, pain sensitivity has previously been evaluated exclusively in cross-sectional studies. It has therefore not been possible to answer the crucial question as to whether the increased pain sensitivity is primary or secondary to the headache. Finally, the development of brain imaging techniques over the last decades has made it possible to study the supraspinal processing of pain in a more direct manner. On this background the aims of the present thesis were to evaluate pain perception in the general population; to explore whether altered pain sensitivity is a primary or a secondary phenomenon to the headache; and to investigate supraspinal processing of muscle pain in chronic tension-type headache. The thesis is based on a cohort study addressing the first two aims and an experimental high-density EEG brain mapping study addressing the third aim.

The cohort study was a combined cross-sectional and follow-up study designed to replicate a population-based study of primary headache disorders and pain perception conducted in Denmark in 1989. The study included a headache interview, a general and neurological examination and measurements of pericranial muscle tenderness, pressure pain threshold, and stimulus response function for pressure versus pain. In agreement with what has been found in patients from headache clinics it was demonstrated that

increased tenderness is also a prominent finding in subjects with tension-type headache in the general population. Increasing slope and displacement towards lower pressures of the stimulus-response function were found in the following order: no headache, migraine, frequent episodic tension-type headache, chronic tension-type headache. The displacement was closely associated with frequency of headache. In agreement with what has been found in patients from headache clinics the stimulus-response function tended to be qualitatively altered in subjects with frequent headache. This suggests, for the first time in a population-based study, that there is a close relation between altered pain perception and chronification of headache which is most likely explained by central sensitization.

In the 12-year follow-up study it was found that subjects who developed frequent episodic tension-type headache had increased pericranial myofascial tenderness but normal general pain sensitivity at follow-up, while subjects who developed chronic tension-type headache had normal pain sensitivity at baseline but had developed increased central pain sensitivity at follow-up. This demonstrates that increased pain sensitivity in tension-type headache is a consequence of frequent headache, not a risk factor. The study is thereby the first to substantiate the hypothesis on central sensitization in a longitudinal study.

In the experimental high-density EEG brain mapping study significant reduction in magnitude during and after induced tonic muscle pain was found in controls at the P200 dipole in response to both the lst and 5th stimulus in the train. In contrast, there were no differences between the conditions in patients. No consistent difference was found in localization or peak latency of the dipoles. The reduction in magnitude during and after induced tonic muscle pain in controls but not in patients may be explained by impaired inhibition of the nociceptive input in patients. This may be the first direct evidence that the supraspinal response to muscle pain is abnormal in patients with chronic tension-type headache.

In conclusion, the present thesis contributes to our understanding of the central mechanisms involved in the chronification of tension-type headache which, hopefully, will lead to better prevention and treatment of chronic tension-type headache in the future.

Kenneth Beri Ploug

KATP channels in intracranial arteries – implications for migraine pathophysiology

A PhD thesis based on the following works:

Ploug KB, Edvinsson L, Olesen J, Jansen-Olesen I. Pharmacological and molecular comparison of K (ATP) channe/s in rat basilar and middle cerebralarteries. *Eur J Pharmacol.* 2006; 553: 254–262.

Ploug KB, Boni U, Baun M, Hay-Schmidt A, Olesen J, Jansen-Olesen I. K(ATP) channelexpression and pharmacological in vivo and in vitro studies of the K(ATP) channel blocker PNU-37883A in rat middle meningeal arteries. *Br J Pharmacol.* 2008; l54: 72–81.

Ploug KB, Sørensen MÅ, Strøbech L, Klaerke DA, Hay-Schmidt A, Sheykhzade M, Olesen J, Jansen-Olesen I. *K(ATP} channels in pig and human intracranial arteries. Eur J Pharmacol.* 2008; 601: 43–49.

Background and purpose: Migraine is a neurovascular disorder which world-wide affects around 15% of the general population. The socioeconomic implications are extensive with considerable impact on productivity and quality of life. Dilatation of cerebral and meningeal arteries causes a throbbing, migraine-like pain, indicating that these structures are involved in migraine pathogenesis. Vascular adenosine 5'-triphosphate-sensitive K+ (KATP) channels are important in the regulation of cerebrovascular tone. Clinical trials using synthetic KATP channel openers (such as levcromakalim and pinacidil) indicate that KATP channel opening may cause migraine headache by dilating intracranial arteries. KATP channels gather to form tissue-specific octameric complexes of four ion pore forming subunits (Kir6.1 or Kir6.2) associated with outer regulatory subunits (SURI, SUR2A or SUR2B). We studied the KATP channel subunit expression profile in the rat/pig/human middle meningeal artery (MMA), the rat/pig middle cerebral artery (MCA) and the rat basilar artery (BA). We also examined the in vitro vasodilatory effects of several KATP channel openers (such as levcromakalim and pinacidil) in rat/pig MMA, rat/pig MCA and rat BA. Furthermore, wc studied the involvement of endothelial versus smooth muscle KATP channels after application of KATP channel openers to isolated rat MCA and BA. Finally, we examined the potential inhibitory effects of the Kir6.1 specific KATP channel blocker PNU-37883A on KATP channel opener-induced relaxation of rat MMA (in vivo and in vitro) and pig MMA/MCA (in vitro).

Experimental approach: mRNA and protein expression of KATP channel subunits in the intracranial arteries were studied by RT-PCR/qPCR and Western blotting, respectively. The in vivo and in vitro pharmacological responses of intracranial arteries to KATP channel drugs were studied in the genuine closed cranial window model and in wire myographs, respectively. Endothelial versus smooth muscle KATP channel activity was examined in a pressure myograph that allows drugs to be applied intraluminally or abluminally to isolated blood vessels.

Key Results: Expression studies indicate that Kir6.1/SUR2B is the major KATP channel complex in the examined intracranial arteries of rat, pig and human. Application of KATP channel openers to rat and pig intracranial

arteries in vitro caused a concentration-dependent vasodilatation with an order of potency that supports the presence of functional SUR2 subunits rather than functional SUR1 subunits. Vasodilatory effects were only apparent when administering KATP channel openers abluminally to isolated rat MCA and BA in the pressure myograph. PNU-378 83A potently inhibited the vasodilatory effects of KATP channel openers in rat MMA (in vivo and in vitro) and pig MMA/MCA (in vitro).

Conclusions and implications: Our combined molecular and pharmacological studies suggest that Kir6.1/SUR2B is the major functional KATP channel complex in intracranial arteries of rat, pig and human, and study results justify the use of a rat as a model to study KATP channel characteristics in intracranial arteries of humans. Pharmacological in vitro studies demonstrate bot h the vasodilatory effects of KATP channel openers in rat and pig intracranial arteries as well as shared KATP channel characteristics between rat and pig intracranial arteries. Furthermore, we show the potent blocking effects ofPNU-37883A on KATP channel opener induced relaxation of rat MMA (in vivo and in vitro) and pig MMA/MCA (in vitro). We provide the molecular evidence that the Kir6.1 blocker PNU-37883A acts on the examined intracranial arteries. Our studies in rat MCA and BA suggest that the direct vasodilatory effects of KATP channel openers rely on smooth muscle KATP channels and not on endothelial KATP channels. The vasodilatory effects of KATP channel openers observed in rat and pig intracranial arteries may be the cause of their strong headache-generating effects in humans. We suggest that a specific blocker of the Kir6.1 or SUR2B KATP channel subunit in cerebral and meningeal arteries to promote vasoconstriction inhibit vasodilatation may be a new strategy to treat migraine headache.

Troels Wienecke

Possible role of prostanoids in migraine and other headaches evaluated in an experimental human

A PhD thesis based on the following works:
Wienecke T, Olesen J, Oturai PS, Ashina M. Prostacyclin (epoprostenol) induces headache in healthy subjects. *Pain* 2008; 139: 106–116.
Wienecke T, Olesen J, Oturai PS, Ashina M. Prostaglandin E_2 (PGE_2) induces headache in healthy subjects. *Cephalalgia* 2009; 29: 509–519.
Wienecke T, Olesen J, Ashina M. Discrepancy between strong cephalic arterial dilatation and mild headache caused by prostaglandin D_2 (PGD_2). *Cephalalgia* 2011; 31(1): 65–76.
Wienecke T, Olesen J, Ashina M. Prostaglandin I_2 (epoprostenol) triggers migraine-like attacks in migraineurs. *Cephalalgia* 2010; 30: 179–190.

Prostanoids are products of the arachidonic acid cascade and highly relevant to the pathophysiology of migraine without aura. Inhibition of prostanoid synthesis is effective in the treatment of headache and migraine without aura, and headache is reported as adverse event during infusion of Prostaglandin I_2, Prostaglandin E_2 and prostaglandin D_2.

The aim of the present thesis was to explore the headache eliciting effect of the prostanoids PGI_2 (prostacyclin), prostaglandin E_2 (PGE_2) and prostaglandin D_2 (PGD_2) in healthy subjects. In addition, explore the possible migraine triggering effects of PGI_2 in migraineurs without aura.

PGI_2, PGE_2 and PGD_2 were infused intravenously in placebo controlled double blind cross- over studies. Headache intensity and associated symptoms were recorded according to the International Headache Society (IHS). Velocity in the middle cerebral artery and diameter in the superficial temporal artery (STA) and radial artery (RA) were measured with ultrasonography and regional cerebral blood flow (rCBF) using ^{133}Xe inhalation and Single Photon Emission Computerized Tomography (SPECT) to estimate relative changes in diameter of the middle cerebral artery (MCA). PGI_2 and PGE_2 induced a mild headache (median VRS 1–2) associated with vasodilatation in healthy subjects. In contrast to PGI_2 and PGE_2, PGD_2 did not trigger a moresevere headache despite a large and prolonged vasodilatation. PG_2 triggered a migraine like headache with associated dilatation in migraineurs without aura and 75% reported the headache to mimic a spontaneous migraine attack without aura. These findings support a role for prostanoids in the pathophysiology of migraine without aura. Sensitization of nociceptors might be central in the pathophysiology of migraine without aura.

Jakob Møller Hansen

Familial hemiplegic migraine – an experimental genetic headache model

A PhD thesis is based on the following works:
Hansen JM, Thomsen LL, Olesen J, Ashina M. Familial Hemiplegic Migraine type 1 shows no hypersensitivity to nitric oxide. *Cephalalgia* 2008; 28: 496–505.
Hansen JM, Thomsen LL, Marconi R, Casari G, Olesen J, Ashina M. Familial hemiplegic migraine type 2 does not share hypersensitivity to nitric oxide with common types of migraine. *Cephalalgia* 2008; 28: 367–375.

Hansen JM, Thomsen LL, Olesen J, Ashina M. Calcitonin gene-related peptide does not cause the familial hemiplegic migraine phenotype. *Neurology* 2008; 71(11): 841–847.
Hansen JM, Bolla M, Magis D, de Pasqua V, Ashina M, Thomsen LL, Olesen J, Schoenen J. Habituation of evoked responses is greater in patients with familial hemiplegic migraine than in controls: a contrast with the common forms of migraine. *Eur J Neurol.* 2011; 18: 478–485.

Familial hemiplegic migraine (FHM) is a rare, dominantly inherited subtype of migraine with aura, where hemiplegia occurs during the aura phase. Mutation screening of families with FHM has revealed a range of different mutations. The mutated FHM genes code for ion transport proteins. Abnormal cortical excitability due to dysfunctional ion-channels might facilitate cortical spreading depression (CSD) and thereby migraine aura and migraine headache. Genotyped FHM patients offer us the chance to study the interplay between genotype and phenotype and may be regarded as a genetic migraine model. FHM studies might open for a better understanding of the molecular migraine pathology, and potentially help to unravel the pathogenesis of the more common migraine forms. We have therefore studied genotyped FHM patients to understand the effect of genotype on the response to migraine provoking substances. We show here that two known migraine triggers failed to induce more migraine aura or migraine headache in FHM-patients than in healthy controls, thus indicating that the FHM genotype does not confer hypersensitivity to these migraine triggers. This has implications for our understanding of the headache mechanisms and raises the question whether FHM shares neurobiological background with the common types of migraine. The aims of the present thesis were to test the hypothesis that FHM mutations might be associated with hypersensitivity to known migraine triggers and, thereby, share pathophysiological pathways with the common types of migraine, but our results disprove this hypothesis. Thus, FHM seems very different from MO and MA, both genetically and pathophysiologically. The fact that FHM genes regulate ion homeostasis cannot be extrapolated to the common types of migraine.

Kim Lindelof

Antinociceptive mechanisms in chronic tension-type headache

A PhD thesis based on the following works:
Lindelof K, Bendtsen L. Memantine for prophylaxis of chronic tension-type headache – a double-blind, randomized, crossover clinical trial. *Cephalalgia* 2009; 29: 314–321
Lindelof K, Ellrich J, Jensen R, Bendtsen L. Central pain processing in chronic tension-type headache. *Clin Neurophysiol.* 2009; 120: 1364–1370.
Lindelof K, Jung K, Ellrich J, Jensen R, Bendtsen L. Low-frequency electrical stimulation induces long-term depression in patients with chronic tension-type headache. *Cephalalgia.* 2010; 30: 860–867

Chronic tension-type headache (CTTH) patients are more sensitive to nociceptive stimuli than healthy individuals. The generalized hyperalgesia may be caused by central sensitization, e.g. in particular deficient descending inhibition. The aim of this thesis was to investigate antinociceptive properties in CTTH patients to elucidate the pathophysiological mechanisms and potential treatment options for this widespread disorder.

In animal pain models, central sensitisation can be counteracted by NMDA-receptor antagonists and they have been suggested to have an effect in similar chronic pain conditions as fibromyalgia patients. Therefore the prophylactic effect of the NMDA-receptor antagonist, memantine, was investigated in chronic tension-type headache patients. This study was designed as a double-blind, placebo controlled, crossover trial with 40 CTTH patients. There was no statistically significant effect for the primary endpoint, area-under-the-headache curve (intensity x duration), in the whole group, while there was a significant reduction in the same parameter in the sub-group of women. In addition, memantine reduced pain intensity significantly in CTTH patients. It was concluded that memantine does not have a clinically significant effect in CTTH, while the modest analgesic effect indicated a pathophysiological significance. Future NMDA-antagonists with higher efficacy could be of major interest with regard to the pathophysiology and future treatment of CTTH and other chronic pain disorders.

Disease models are important, both to test potential treatments and to understand the pathogenesis of an illness. Hypertonic saline injections into the neck muscles in humans have resulted in pain with a distribution similar to CTTH. A similar experimental design investigating brainstem reflex changes in mice has been suggested as a potential animal model for CTTH. In our second study we therefore combined these 2 models. The aim was to investigate whether the blink reflex was modulated differently in patients with CTTH and healthy controls during tonic pain to gain insight into central pain mechanisms in this disorder. In 20 CTTH patients and 20 healthy volunteers, pain perception of electrical stimuli to the forehead and the blink reflex was recorded before, during and after infusion of hypertonic saline into the neck muscles. The rating of painful stimuli was significantly higher in CTTH patients than in healthy volunteers. The relative change of the blink reflex integral immediately after hypertonic saline infusion was significantly smaller in CTTH patients on the

contralateral but not on the ipsilateral side compared to healthy volunteers. A combined homotopic and heterotopic effect of the conditioning pain onto the blink reflex could account for this finding. It was concluded that there was no robust difference in BR integral after conditioning tonic neck pain between CTTH patients and healthy volunteers, but CTTH patients were more sensitive to nociceptive stimuli.

Deficiencies in the endogenous pain inhibitory system have been suggested as a possible explanation of central sensitization and CTTH. Long-term depression (LTD) of pain was recently demonstrated in healthy volunteers by repeated low-frequency electrical stimulation (LFS). The aim of our third study was to test if these inducible antinociceptive mechanisms were intact in CTTH patients and whether pain mechanisms were modulated at the level of the brain stem. In 20 CTTH patients and 20 healthy controls LFS was delivered to the forehead and pain ratings and blink reflexes were recorded before and after LFS. After LFS, significant reductions of the pain score of the electrical stimuli and of the integral of the blink reflex about 20–30% compared to baseline were seen both in CTTH patients and healthy volunteers. This inhibitory effect lasted throughout the post-LFS study period of 1 hour. It was concluded, that a significant and stable pain inhibition (LTD) can also be induced in CTTH patients by LFS and indicate that chronic TTH can be modified by external simple stimuli. The corresponding decrease in the nociceptive brainstem reflex indicates that pain was modulated at the level of the brainstem. It is possible that low-frequency electrical stimulation can be further developed to a highly needed alternative to pharmacological treatment for chronic pain patients.

This thesis contributes to the understanding of the pathophysiology of chronic tension-type headache and may lead to forward treatment possibilities.

Henrik Winther Schytz

Investigation of carbachol and PACAP38 in a human model of migraine

A PhD thesis based on the following works:
Schytz HW, Wienecke T, Oturai PS, Olesen J, Ashina M. The cholinomimetic agent carbachol induces headache in healthy subjects. *Cephalalgia* 2009; 29: 258–268.
Schytz HW, Wienecke T, Olesen J, Ashina M. Carbachol induces headache, but not migraine-like attacks, in patients with migraine without aura. *Cephalalgia* 2010; 30: 337–345.
Schytz HW, Birk S, Wienecke T, Kruuse C, Olesen J, Ashina M. PACAP38 induces migraine-like attacks in patients with migraine without aura. *Brain* 2009;132: 16–25.
Schytz HW, Holst H, Arendt-Nielsen L, Olesen J, Ashina M. Cutaneous nociception and neurogenic inflammation evoked by PACAP38 and VIP. *J Headache Pain* 2010; 11: 309–316.

The parasympathetic signalling molecules acetylcholine, pituitary adenylate cyclase activating peptide-38 (PACAP38) and vasoactive intestinal peptide (VIP) may be released from parasympathetic fibres and activate sensory nerve fibres during migraine attacks. Recently, it was shown that VIP does *not* induce migraine-like attacks in migraine patients. Interestingly, PACAP38 activates the same VPAC receptors as VIP, but also specifically activates the PAC_1 receptor.

The present thesis includes four double-blind placebo-controlled crossover studies aimed to explore the role of acetylcholine, PACAP and VIP in migraine and head pain. In study I-III we investigated acetylcholine, via the analogue carbachol, and PACAP38 in a human model of migraine. In study IV we studied if PACAP38 and VIP might induce central sensitization, neurogenic inflammation and mast cell degranulation in a cutaneous model of acute pain.

Study I-II showed that carbachol induced short lasting mild headache and moderate cephalic vasodilatation in both healthy volunteers and migraine patients, but did not induce migraine-like attacks. In study III PACAP38 induced headache in healthy subjects and delayed migraine-like attacks in migraine patients as well as sustained dilatation of cephalic vessels. In study IV VIP and PACAP38 evoked skin pain, central sensitization, neurogenic inflammation and mast cell degranulation, but VIP showed to be more potent than PACAP38 in inducing neurogenic inflammation and mast cell degranulation.

In conclusion, we found that carbachol infusion was not a good model for experimental migraine provocation, probably because the maximal dose was insufficient to produce enough nitric oxide to trigger migraine. PACAP38 infusion is a new pathway for migraine induction and the results from study IV suggests that neurogenic inflammation and mast cell degranulation are unlikely to cause PACAP38 induced migraine. The present thesis contributes to our knowledge on migraine pathophysiology and suggests PAC_1 receptor antagonism as a new target for migraine treatment.

Helle Wulf-Johansson

Expression of BKca channels in the trigeminovascular system – relevance for migraine

A PhD thesis based on the following works:
Wulf-Johansson H, Hay-Schmidt A, Poulsen AN, Klaerke DA, Olesen J, Jansen-Olesen I. Molecular studies of BK_{Ca}

channels in intracranial arteries: presence and localization. *Cell and Tissue Research* 2008; 334(3): 359–369.
Wulf-Johansson H, Hay-Schmidt A, Poulsen AN, Klaerke DA, Olesen J, Jansen-Olesen I. Molecular investigations of BK_{Ca} channels and the modulatory β-subunits in porcine basilar and middle cerebral arteries. *Journal of Molecular Histology* 2009; 40(2): 87–97.
Wulf-Johansson H, Hay-Schmidt A, Poulsen AN, Klaerke DA, Olesen J, Jansen-Olesen I. Expression of BK_{Ca} channels and the modulatory β-subunits in the rat and porcine trigeminal ganglion. *Brain Research* 2009; 1292: 1–13.
Wulf-Johansson H, Hay-Schmidt A, Poulsen AN, Klaerke DA, Olesen J, Jansen-Olesen I. Localization of BK_{Ca} channels and effect of BK_{Ca} channels on CGRP release in the rat trigemino-neuronal pathway. *Neuroscience* 2010.

The migraine pathophysiology is divided into two hypotheses, e.g. the vascular and the neuronal pathways, thus it seems to be a combined disease of vascular events together with neuronal involvement. Dilation of large arteries and meningeal arteries causes a throbbing, unilateral migraine-like pain in patients. For that reason, dilatation of blood vessels is believed to play a major role in headache pathogenesis. Central trigeminal pain pathways include the TG and the TNC which transmit nociceptive pain signals from the peripheral to the higher brain centre where the pain is perceived. Malfunction of these structures may be implicated in the initiation of migraine causing hyperexcitable neurons. BK_{Ca} channels play an important role in the regulation of cerebral artery diameter and may therefore be important in cerebrovascular pathogenesis such as migraine and stroke. Most studies examining BK_{Ca} channels in cerebral arteries have used electrophysiology and pharmacological approach. BK_{Ca} channels are also expressed in neurons where they modulate neuronal excitability and neurotransmitter release. Thus, BK_{Ca} channels may be essential in neurological diseases. To gain more insight into the presence of BK_{Ca} channels and the modulatory β-subunits in the trigeminovascular system, we performed a characterization study of BK_{Ca} channel mRNA and protein expression profile in migraine relevant tissues such as 1) cerebral arteries including rat/porcine BA, rat/porcine MCA and rat MMA 2) the rat/porcine TG and 3) the rat TNC using molecular biology methods. The general aims of this thesis were:

To investigate the expression profile of BK_{Ca} channel mRNA and protein in rat BA, MCA and MMA (article I).

To investigate the expression profile of BK_{Ca} channel mRNA and protein in porcine BA and MCA and to study the modulatory β-subunit mRNA and protein composition (article II).

To investigate the expression profile of BK_{Ca} channel mRNA and protein in rat and porcine TG and to study the modulatory β-subunit mRNA and protein composition in TG (article III).

To investigate the expression profile of BK_{Ca} channel mRNA and protein in rat TG and rat TNC and to investigate the modulatory β-subunit protein composition in the TG and the TNC. Furthermore, the role of BK_{Ca} channels in release of CGRP from TG and TNC were investigated by using the BK_{Ca} channel blocker iberiotoxin and the BK_{Ca} channel opener NS11021 (article IV).

Results from the vascular studies. BK_{Ca} channel mRNA expression was detected in all arteries studied. Higher expression patterns of BK_{Ca} mRNA were observed in BA as compared to MCA and MMA. At the protein level BK_{Ca} channels were more expressed in BA as compared to MCA and MMA. The BK_{Ca} mRNA was localized to the smooth muscle cells and immunofluorescent imaging also verified the BK_{Ca} channel protein to the smooth muscle cells using the α-actin marker. The modulatory β1-subunit was detected in BA and MCA.

Results from the neuronal studies. BK_{Ca} channel mRNA expression was detected in TG and TNC. No differences were found at the mRNA level comparing TG and TNC. The BK_{Ca} channel protein was shown in TG and TNC with higher protein expression pattern in TNC as compared to TG. Histochemistry studies showed the localization of BK_{Ca} channel protein in the perikarya of TG and TNC body cells. The modulatory β2- and β4-subunit was detected in TG and TNC. However, they were more abundant in TNC than in TG. We studied a possible functionality of BK_{Ca} channels on CGRP release from TG and TNC in vitro and found that the BK_{Ca} channel blocker iberiotoxin caused an increase in CGRP release from the TNC. The response was attenuated by pre-treatment with the BK_{Ca} channel opener NS11021. No effect was observed in TG.

The findings of the BK_{Ca} channel in both vascular and neuronal tissues may suggest a possible role of BK_{Ca} channels in the trigeminovascular nociceptive pain pathway and be an advantage in future therapeutic drug development to migraine research. We suggest that a BK_{Ca} channel blocker may be used to counteract the vascular dilatation whereas a BK_{Ca} channel opener may be effective in the suppression of hyperexcitable sensory neurons.

Maren Skau

Idiopathic intracranial hypertension – New aspects of diagnosis and handling

A PhD thesis based on the following works:
Skau M, Sander B, Milea D, Wegener M, Jensen R. OCT for optic disc evaluation in idiopathic intracranial hypertension. *Graefe's Arehive for Clinical and Experimental Ophthalmology* 201 1; 249: 723–730.

Skau M, Sander B, Milea D, Jensen R. Disease activity in idiopathic intracranial hypertension. A 3-month follow-up study. *J Neurology* 2011; 249: 723–730.

Idiopathic intracranial hypertension (IIH) is a clinical condition of elevated intracranial pressure of still unknown pathogenesis typically seen in obese women. Untreated IIH may lead to severe visual loss and blindness resulting from post papilledema optic atrophy. Identification of papilledema and close follow-up of treatment response are therefore mandatory to reduce the risk of a poor visual outcome.

On this background, the aim of the present thesis was to explore the ability of optical coherence tomography (OCT) to identify and quantify peripapillary retinal changes related to papilledema and to describe these changes in relation to intracranial pressure and body mass index. In addition, to search for a diagnostic biological marker of IIH.

A controlled cross-sectional cohort of newly diagnosed and chronic IIH patients was evaluated by lumbar CSF pressure measurement, OCT, autoperimetri and analysis of natriuretic pro-peptides in plasma and cerebrospinal fluid. The newly diagnosed IIH patients also constituted a longitudinal cohort for a 3-month follow-up study of OCT, autoperimetri, CSF pressure and natriuretic pro peptides.

The ability of OCT to quantify and follow-up optic disc changes in papilledema and to predict the presence of intracranial hypertension was demonstrated. Results suggested that the conventionally used peripapillary OCT estimate of the retinal nerve fiber layer thickness may underestimate the thickness of the retinal nerve fiber layer for increasing papilledemas. Instead, the peripapillary total retinal thickness may be a more robustestimate of optic disc swelling.

A significantly reduced plasma concentration of pro CNP was identified in IIH patients. The pathophysiological significance of this remains to be elucidated in future studies, but findings raised the hypothesis that pro CNP may serve as a diagnostic biological marker for IIH.

A linear association between the proportional change in body mass index and the proportional change in CSF pressure among newly diagnosed UH-patients during a 3-month period was found. A substantial benefit of weight loss for disease remission is therefore hypothesized.

In conclusion, the present thesis provides promising results for improving the diagnostic process and the treatment of IIH.

Anne Werner Hauge

Migraine with aura triggers and treatment

A PhD thesis based on the following works:

Hauge AW, Kirchmann M, Olesen J. Trigger factors in migraine with aura, *Cephalalgia* 2010; 30: 346–353.
Hauge AW, Kirchmann M, Olesen J. Characterization of consistent triggers of migraine with aura, *Cephalalgia* 2011; 31: 416–438.
Hauge AW, Asghar MS, Schytz HW, Christensen K, Olesen J. Effects of tonabersat on migraine with aura: a randomised, double-blind, placebocontrolled erossover study, *Lancet Neurology* 2009; 8: 718–723
Hauge AW, Hougaard A, Olesen J. On the methodology of drug trials in migraine with aura, *Cephalalgia* 2011; 31: 416–438.

Cortical spreading depression (CSD) is thought to be the pathophysiological mechanism underlying the migraine aura.

Whether CSD is also part of an attack of migraine without aura (MO) is much less likely, although controversial. To further expand our knowledge of the difference; and similarities of migraine with aura (MA) and MO, studies focusing on the individual subtypes of migraine are necessary.

The aims of this thesis were to investigate the prevalence of trigger factors in patients with MA, to investigate the efficacy of tonabersat, an inhibitor of CSD, in the preventive treatment of MA, and to evaluate the methodology of drug trials in MA. MA patients report trigger factors with a very high frequency. Stress, in particular work related stress, was the most prevalent trigger factor in MA patients. Patients having attacks of both MA and MO reported almost identical trigger factors for their attacks. Treatment with tonabersat showed a statistically significant reduction in number of attacks of aura, whereas number of days with migraine headache was not statistically significantly reduced. In trials with MA patients, a very detailed description of the individual attacks is required, in order to differentiate attacks of MA and MO by classifying all attacks in accordance to the International Classification of Headache Disorders 2. Endpoints related to the aura are desirable.

This thesis supports that CSD is the pathophysiological mechanism underlying the migraine aura, and that CSD not is likely to take part in an attack of MO. MA and MO are much alike with regard to trigger factors, and whether MA and MO are different diseases or just variants of the same disease remains to be shown.

Mohammad Sohail Asghar

Effects of glyceryl trinitrate and calcitonin gene-related peptide on BOLD signal and arterial diameter: methodological studies by fMRI and MRA

A PhD thesis based on the following works:

Asghar MS, Hansen AE, Pedersen S, Larsson HB, Ashina M. Pharmacological modulation of the BOLD response – a study of acetazolamide and glyceryl trinitrate in humans. *J Magn Reson Imaging* 2011; 34: 921–927
Asghar MS, Hansen AE, Kapijimpanga T, van der Geest RJ, van der Koning P, Larsson HB, Olesen J, Ashina M. Dilation by CGRP of middle meningeal artery and reversal by sumatriptan in normal volunteers. *Neurology* 2010: 75: 1520–1526
Asghar MS, Hansen AE, Larsson HB, Olesen J, Ashina M. Effect of CGRP and sumatriptan on the BOLD response in visual cortex. *J Headache Pain.* 2012;13: 159–66.

Over the last decades MRI has proved to be very useful in the field of drug development and drug discovery. Pharmacological MRI (phMRI) explores the interaction between brain physiology, neuronal activity and drugs. The BOLD signal is an indirect method to investigate brain activity by way of measuring task related hemodynamic changes. Pharmacological substances that induce hemodynamic changes can therefore potentially alter the BOLD signal and in turn falsely can be interpreted as changes in neuronal activity. It is therefore important to characterize possible effects of a pharmacological substance on the BOLD response per se before that substance can be used in an fMRI experimental setup in order to avoid false positive or false negative results. Furthermore MRI and MRA is useful in determining the vascular site-of-action of vasoactive substances. Four substances; acetazolamide, GTN, CGRP and sumatriptan has been examined in double-blind placebo controlled cross-over studies. The present thesis includes three papers with the aim to determine the site-of-action and to explore the effects of the pharmacological agents on the BOLD signal. Study I showed that acetazolamide depressed the BOLD signal by increasing CBF. GTN is known to increase CBV but had surprisingly no effect on the BOLD signal. This is probably because the GTN induces CBV increase is limited to the large arteries whereas the hemodynamic changes associated with the BOLD signal are anatomically located in the capillaries and venoules. Study II and III showed that systemic administration of CGRP induces immediate headache and dilates the MMA but contrarily to previous belief does not dilate the MCA. Nor does CGRP increase brain activity per se. Sumatriptan ameliorates headache, contracts MMA and marginally contrasts MCA without altering brain activity. In conclusion we found that acetazolamide depresses the BOLD signal while GTN does not alter the BOLD signal. While systemic administration of CGRP or sumatriptan has no direct effects on brain activity in healthy volunteers. Instead it seems that both migraine provoking peptide CGRP and anti-migraine drug sumatriptan exert their actions outside of the BBB. This thesis shows that phMRI is a powerful tool in understanding mechanisms and site-of-action of pharmacological compounds. phMRI could be a useful addition to the existing methods for development of future drugs.

Michael Baun

Characterization of VIP and PACAP-receptors in a trigeminovascular model-relevance for migraine

A PhD thesis based on the following works:
Baun M, Bay-Schmidt A, Edvinsson L, Olesen J, Jansen-Olesen I. Pharmacological characterization and expression ofVIP and PACAP receptors in isolated cranial arteries of the rat. *European Journal of pharmacology* 2011; 670: 186–194.
Baun M, Pedersen MHF, Olesen J, Jansen-Olesen I. Dural mast cell degranulation is a putative mechanism for headache induced by PACAP-38. *Cephalalgia* 2012; 32: 337–345.
Baun M, Ramachandran R, Christophersen DV, Amrutkar D, BaySchmidt A, Gupta S, Olesen J, Jansen-Olesen I. VIP and PACAP co-localization and functional interactions with NOS and CGRP. *Cephalalgia* 2012; 31: 181–189.

Neuropeptides vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating peptide-38 (PACAP-38) have previously both been assessed for migraine-inducing properties in a human provocation model. Though eliciting comparable vasodilating properties, only PACAP-38 reliably triggered migraine attacks. VIP has a high affinity to $VPAC_1$ and $VPAC_2$ receptors. PACAP-38 has a similar high affinity to $VPAC_1$ and VPAC2, but furthermore to a dedicated PACAP-receptor, PAC_1. PACAP exists naturally in two isoforms, the most abundant PACAP-38 and a C-truncated 27-amino acid form PACAP-27. The two forms have similar affinities to the three known receptors. The high similarity between VIP and PACAP-38 combined with their different migraine-inducing potential gives a rare opportunity to isolate which property causes one to induce migraine and the other not. This can lead to new insights in migraine pathophysiology.

At the present, there are different hypothesis for neuropeptide-induced migraine. This thesis is divided in three projects all focusing on the VIP/PACAP peptides but addressing different anatomical compartments and mechanisms of action. Through this approach, it was attempted to elucidate differences in action that might shed light on the basis of PACAP-induced migraine.

Paper I focuses on the vascular compartment, through *in vitro* studies of vasodilating properties of VIP, PACAP-27 and PACAP-38, blocked with selective receptor antagonists and expression studies. The tissues used in this paper were migraine-relevant intracranial arteries from the rat: Basilar artery (BA), middle cerebral artery (MCA) and middle meningeal artery (MMA). The functional assays wire myography and perfusion myography were combined

with *in situ* hybridization for expression studies. Neither VIP nor PACAP induced relaxation of the MMA in the wire myograph. Pure PAC_1-agonist maxadilan induced no relaxation in any of the tested vessels.

Luminal administration of VIP in the perfusion myograph did not cause relaxation, excluding an endothelial mechanism of action. Potency of the pure agonists was approximately equal, with VIP being slightly more potent in the BA. Experiments with the $VPAC_1$ antagonist PG 97-269 and the VPAC2 antagonist PG 99-265 indicated that relaxation induced by VIP and PACAP was mediated mainly by the $VPAC_1$ receptor and to a lesser degree by the VPAC2 receptor. *In situ* hybridization demonstrated the presence of mRNA transcripts of all three receptors in the smooth muscle cells of the vessels. The conclusions are that PACAP is not a stronger vasodilator than VIP in tested intracranial arteries of the rat, and that VPAC1 is the dominating receptor in mediating vasodilatation in intracranial vessels induced by VIP and PACAP. Thus, the headache-inducing properties of PACAP compared to VIP cannot be explained through a purely vascular hypothesis.

Paper II examines the effect of neuropeptides on mast cells of the rat. Mast cells are widely present in the perivascular areas of the dura mater and release inflammatory mediators on activation. Extracted peritoneal mast cells of the rat and intact dura mater of the rat in hemisected skull preparation were used as models. We investigated the degranulating effects of naturally occurring peptides VIP, PACAP-27 and PACAP-38 as well as N-truncated fragments of PACAP-38. In attempt to determine which intracellular pathway facilitated degranulation, transduction mechanism inhibitors for adenylyl cyclase (AC) and phospholipase C (PLC) were co-administered with the agonists. It was found that PACAP-38 and N-truncated fragments up to and including PACAP(I6-38) induce a much stronger degranulatory response on peritoneal mast cells than PACAP-27 and VIP. The EC_{50} for "strong degranulators" (PACAP-38, PACAP(6-38) and PACAP(I6-38) was on average approximately 30 times lower than for the "weak degranulators" (VIP, PACAP-27 and PACAP(28-38)). The degranulation was significantly inhibited by co-treatment with PLC inhibitor, but was not affected by AC inhibition. In the intact dura mater, the same order of potency for the natural peptides, PACAP38 > PACAP-27 = VIP, was observed. In conclusion, the difference in headache-inducing potential between PACAP-38 and VIP may be explained by mast cell activation by PACAP-38.

Paper III investigates some of the putative mechanisms for peptide-induced migraine induction in the migraine-related tissues trigeminal ganglion (TG), trigeminal nucleus caudalis (TNC) and dura mater of the rat. In these tissues, we performed assays for nitric oxide (NO) induction and calcitonin gene related peptide (CGRP) release against VIP and PACAP-38. Expression of relevant receptors was explored by qPCR. For nitric oxide synthases, CGRP and PACAP, co-localization studies were done by immunostaining. For PACAP-38 and not the other peptids investigated, a concentrationdependent release of CGRP was observed in the TNC. mRNA for the PAC 1 receptor was expressed in all tissues, with TNC > TG > dura mater. mRNA for VPAC2 was most expressed in the TG. No significant effect on nitric oxide synthase activity was observed.

In conclusion, the most profound differences observed in the employed models were the effects of the peptides on mast cells. This suggests that degranulation of mast cells may be among the causative factors for PACAP-induced migraine. Studying these mechanisms in depth may eventually yield novel treatment options for migraine.

Maria Antonova

Prostaglandins and prostaglandin receptor antagonism in migraine

A PhD thesis based on the following works:
Antonova M, Wienecke T, Olesen J, Ashina M. Prostaglandin E (2) induces immediate migraine-like attack in migraine patients without aura. *Cephalalgia* 2012; 32: 822–833.
Antonova M, Wienecke T, Maubach K, Thomas E, Olesen J, Ashina M. The pharmacological effect of BGC20-1531, a novel prostanoid EP4 receptor antagonist, in the prostaglandin E2 human model of headache. *J Headache Pain* 2011; 12: 551–559.
Antonova M, Wienecke T, Olesen J, Ashina M. Pro-inflammatory and vasoconstricting prostanoid PGF2α causes no headache in man. *Cephalalgia* 2011; 31: 1532–1541.

Human models of headache may contribute to understanding of prostaglandins' role in migraine pathogenesis. The current thesis investigated the migraine triggering effect of prostaglandin (PGE_2) in migraine patients without aura, the efficacy of a novel EP4 receptor antagonist, BGC20-1531, in prevention of PGE_2-induced headache and the ability of prostaglandinF2a (PGF2a) to trigger headache without any vasodilatation in healthy volunteers. All studies were designed as a double-blind, placebo-controlled, cross-over experiments, where PGE2/$PGF_{2\alpha}$ or saline were infused over 20–25 min. In study with EP4 receptor antagonist healthy volunteers were pre-treated with two different dosages of BGC20-1531 or placebo followed by PGE2 infusion over 25 min. The headache data were collected during the whole study, whereas the possible vascular changes were measured during the in-hospital phase of 1.5 h.

The infusion of PGE_2 caused the immediate migraine - like attacks and vasodilatation of the middle cerebral artery in migraine patients without aura. The highly specific and potent EP4 receptor antagonist, BGC20-1531,

was attenuated PGE2-induced headache and vasodilatation of both intra- and extra-cerebral arteries. The intravenous infusion of $PGF_{2\alpha}$ did not induce headache or statistically significant vasoconstriction of cerebral arteries in healthy volunteers. Novel data on PGE_2-provoked immediate migraine-like attacks suggest that PGE2 may be one of the important final products in the pathogenesis of migraine. The lack of efficacy of EP4 receptor antagonist suggests that a single receptor blockade is not sufficient to block PGE2 responses; hence EP2 receptor should be investigated as a potential drug target for the treatment of migraine. The absence of headache during the $PGF_{2\alpha}$ infusion demonstrates that vasodilating properties are necessary for the induction of headache and migraine.

Roshni Ramachandran

Possible mechanisms behind GTN induced headache: a pharmacological and molecular approach in elucidating the pain pathway in rats

A PhD thesis based on the following works:
Ramachandran R, Ploug KB, Hay-Schmidt A, Olesen J, Jansen-Olesen I, Gupta S. Nitric oxide synthase (NOS) in the trigeminal vascular system and other brain structures related to pain in rats. *Neurosci Lett*, 2010;484:192–196.
Ramachandran R, Bhatt DK, Ploug KB, Olesen J, Jansen-Olesen I, Hay-Schmidt A, Gupta S. A naturalistic glyceryl trinitrate infusion migraine mode l in the rat. *Cephalalgia* 2012; 32: 73–84.

Nitric oxide (NO) plays a crucial role in primary headache s and is an important factor in triggering migraine attacks. Infusion of the NO donor glyceryl trinitrate (GTN), induces an immediate headache that is followed by a migraine attack 5–6 years later and is a widely accepted human migraine model. The exact neurobiological mechanisms of GTN induced headache are yet to be identified. Improved understanding of headache pathophysiology depends on the availability and validity of appropriate animal models that accurately reflect the human condition and so far there is no consensus on a single animal model in migraine research. Several studies have tried to simulate the GTN human model in anesthetized or in awake rodents using doses of GTN 1 000 times higher than used in humans. The relevance of such toxicological doses to migraine is not certain. Therefore, our aim was to generate an animal model which is similar to the human migraine model. Secondly, we wanted to investigate the possible mechanisms leading to GTN induced headache.

The involvement of endogenous NO production during migraine attacks is substantiated by the anti-migraine effect of the nitric oxide synthase (NOS) inhibitor L-NG-monomethyl arginine citrate (L-NMMA). In Study I the basal levels of NO synthesizing enzymes (NOSs') in the brain tissues relevant to migraine was analyzed. The presence of eNOS and nNOS in most of the migraine relevant structures could suggest a role in inducing and maintaining migraine pain.

Study II and III focused mainly on the development and validation of the animal model by infusing GTN as the migraine inducing substance and measuring the neuronal activation using c-fos as a marker for nociception. This model of awake freely moving rats closely mimics the universally accepted human model of migraine and has a predictive validity since this GTN model responds to sumatriptan treatment. Moreover, we also found that NOS, CGRP and inflammatory systems are involved in GTN induced neuronal activation. Through this study we also predict that olcegepant may induce an inhibitory effect when given as a pre-treatment rather than a post-treatment.

Study IV introduces the possible downstream cascades that may be involved in GTN induced activation and probably in maintaining the effect of GTN for a longer period. We observed that an immediate effect of GTN was seen both in dura and TNC with the activation of p ERK in both the tissues, which was then taken over by TNC in case of long term effects. Several other markers p-CREB/ATF-1 and CamK were also upregulated in the TNC which play an important role in activating the transcription of c-fos, a marker for neuronal activation.

In conclusion, these studies provide a validated animal model for migraine and using pharmacological and biochemical approaches we elucidated the possible mechanisms or pathways involved in the GTN induced neuronal activation in rats. This one provocation model may not be universally useful. It may be necessary to test this model with other migraine provoking substances in order to provide a reliable animal model for migraine.

Maja Myren

Characterization of prostaglandin receptors in the trigeminovascular system – Implications for migraine pathophysiology

A PhD thesis based on the following works:
Myren M, Baun M, Ploug KB, Jansen-Olesen I, Olesen J, Gupta S. Functional and molecular characterization of prostaglandin E_2 dilatory receptors in the rat craniovascular system in relevance to migraine. *Cephalalgia* 2010; 30:1110–1122
Myren M, Olesen J, Gupta S. Pharmacological and expression profile of the prostaglandin I_2 receptor in the rat craniovascular system. *Vascul Pharmacol* 2011; 55: 50–58
Myren M, Olesen J, Gupta S. Prostaglandin E_2 receptor expression in the rat trigeminal-vascular system and

other brain structures involved in pain. *Neurosci Lett* 2012; 506: 64–69

Migraine is one of the most debilitating neurovascular disorders in the Western world and affects around 10% of the general population. The socio-economic implications are enormous with considerable impact on productivity and quality of life. The pathophysiology of migraine involves interaction between cranial blood vessels, perivascular trigeminal innervation and second order neurons in the brain stem. Calcitonin gene-related peptide (CGRP) has been shown to be a key molecule in migraine pathogenesis, and CGRP receptor antagonists are efficient in migraine treatment. In a human provocation model prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) induce headache in healthy volunteers and migraine-like headaches in migraine patients without aura. PGE_2 and PGI_2 are vasodilators and act on the receptors: EP_1-EP_4 and IP, respectively. The aim of this PhD research project was 1) To characterize PGE_2 and PGI_2 dilatory receptors using preclinical animal models of migraine. 2) To explore the expression profile of prostaglandin receptors in the rat brain, in order to locate the most likely 'site of action' for each receptor subtype. 3) To investigate the relation between prostaglandins and CGRP release, as this event seems to be an important part of migraine pathogenesis. The dissertation is based on the four following studies:
Study 1: *Purpose*: to investigate the dilatory effect of PGE_2, *in vivo* and *in vitro*, on intracranial arteries using specific agonists and antagonists, and to quantify the receptor components in the trigeminovascular system. *Findings*: the PGE_2 receptors: EP_2 and EP_4 receptors mediate vasodilation via 3'-5'-cyclic adenosine monophosphate in rat meningeal and cerebral arteries. We show that the EP_4 antagonist, BGC20-1531, is a specific blocker of PGE_2 mediated vasodilation. Real time PCR demonstrated the presence of mRNA transcripts of EP_1-EP_4 receptors in the trigeminovascular system.
Study 2: *Purpose*: to investigate the effect of PGI_2 on intracranial arteries in the rat *in vivo*, and to localize the IP receptor component in the trigeminovascular system. *Findings*: Topical application and intra carotid infusion of iloprost (a stable PGI_2 analogue) caused dilatation of the rat meningeal artery *in vivo*. This effect could be blocked by the IP receptor specific antagonist, CAY10441. We demonstrated the presence of mRNA transcripts of the IP receptor in the trigeminovascular system and this was confirmed at the protein level. Immunostaining revealed the IP receptor localized to the smooth muscle cells of the meningeal, cerebral and basilar arteries of the rat.
Study 3: *Purpose*: to show the localization of EP_1-EP_4 receptors in different areas of the rat brain. *Findings*: the EP_1-EP_4 receptors are ubiquitously expressed in all tested tissues both at mRNA as well as protein levels.
Study 4: *Purpose*: To investigate the brain areas with the highest expression of IP receptor with the aim of targeting drug specific areas. To investigate whether the IP receptor and CGRP co-localize in the trigeminal ganglion (TG) and trigeminal nucleus caudalis (TNC), and to examine the PGI_2 involvement in the release of the migraine essential peptide CGRP in rat dura mater, TG and TNC. *Findings*: the IP receptors are ubiquitously expressed in all tested tissues at mRNA as well as protein levels. High levels of IP receptor staining was found in TG and TNC where it was found co-localized with CGRP. PGI_2 *per se* could not induce iCGRP release. Iloprost significantly attenuated the KCl-induced iCGRP release in rat skull halves, an inhibition that was independent of IP, EP_1 and EP_3.

The findings involving the PGE_2 and PGI_2 in both vascular and neuronal tissues suggest a possible role of the prostaglandin receptors in the trigeminovascular nociceptive pain pathway. The studies performed in this dissertation can add knowledge that can be used in future therapeutic drug development for migraine treatment.

Signe Bruun Munksgaard

Medication-overuse headache – Pain mechanisms and treatment

A PhD thesis based on the following works:
Munksgaard SB, Allena M, Tassorelli C, Rossi P, Katsarava Z, Bendtsen L, Nappi G, Jensen R and the COMOESTAS consortium: What do the patients with medication overuse headache expect from treatment and what are the preferred sources of information? *J. Headache Pain* 2011; 12: 91–96.
Munksgaard SB, Bendtsen L, Jensen RH: Treatment-resistant medication overuse headache can be cured. *Headache* 2012; 52: 1120–1129.
Munksgaard SB, Bendtsen L, Jensen RH: Detoxification of medication-overuse headache by a multidisciplinary treatment programme is highly effective: a comparison of two consecutive treatment methods in an open-label design. *Cephalalgia* 2012; 32: 834–844.
Munksgaard SB, Bendtsen L, Jensen RH: Central sensitization is increased in medication overuse and can be reduced after detoxification: results of a 12 months detoxification study. *Cephalalgia* 201; 33: 444–453.

The aims of this thesis were to evaluate patient expectations to treatment, evaluate the effect of two structured detoxification programmes in patients with previously unsuccessfully treated MOH and investigate pain perception before and in the year after withdrawal.

The outcome of a treatment is related to how the patients' expectations are met. It is therefore important to inform the patient properly on how the treatment is conducted and what outcomes to expect. In collaboration between three specialized headache centres in Italy, Germany and Denmark, we developed a questionnaire to evaluate the expectations to MOH treatment and which sources of information the patients needed and preferred. The patients preferred personal information, but also welcomed internet-based information sources. They had high and in some ways unrealistic expectations to the treatment outcome and seemed eager to participate actively in the treatment since many expected education on how best to handle the headache.

Treatment for MOH is focused on withdrawal from the overused substances. But whether prophylactic treatment should be initiated from withdrawal start or should be postponed to the effect of detoxification is known, is not agreed.

Patients who have previously failed withdrawal are often excluded from MOH studies and consensus lacks whether these patients should be detoxified or should just receive prophylactic medication for their headache.

We investigated the effect of structured detoxification in two different setups and included patients with previously unsuccessfully treated MOH. In programme A, patients received individual information on MOH and prophylactic and acute medication in the first two months of withdrawal. In programme B, patients received multidisciplinary education in groups, but no prophylactic and acute medication in the first two months. Patients in programme B needed less acute and prophylactic medication at the 1-year follow-up and also had fewer unplanned contacts with the physicians compared with programme A. Both programmes proved highly efficient with a 1-year headache frequency reduction of almost 40% and 10% drop-out rate.

Studies of MOH pathophysiology report alterations in pain perception, but a few human and animal studies suggest that the pain perception improves only shortly after detoxification. To investigate a long term course of pain perception after withdrawal for the first time, we followed MOH patients with quantitative sensory testing for a year. The patients were centrally sensitized before withdrawal and did not show temporal summation. After withdrawal, the pain scores gradually decreased over the year towards the pain scores of healthy volunteers. We also demonstrated that temporal summation was seen already two months after withdrawal and it persisted throughout the study.

In summary, the positive results of normalized pain perception and significant improvement after detoxification emphasize the importance of detoxification as treatment for MOH, also in patients who have previously been unsuccessfully treated. Still, studies of MOH pathophysiology are needed especially for overall prevention and for MOH on the basis of tension-type headache. Also properly randomized studies with at least a 1-year follow-up on the effect of prophylactic and acute medication during withdrawal as well as multidisciplinary education are important targets for future prevention and management. Already now, however, it can be concluded that a general information campaign to prevent and identify MOH should be initiated since MOH is a disabling, chronic pain condition, which can and ought to be prevented and treated.

Han Le

The environmental risk factors and co-morbidity in migraine – a population-based study in twins

A PhD thesis based on the following works:
Le H, Tfelt-Hansen P, Russell MB, Skytthe A, Kyvik KO, Olesen J. Co-morbidity of migraine with somatic disease in a large population-based study. *Cephalalgia* 2011; 3: 43–64.
Le H, Tfelt-Hansen P, Skytthe A, Kyvik KO, Olesen J. Association between migraine, lifestyle and socioeconomic factors: a population-based cross-sectional study. *J Headache Pain* 2011; 12: 157–172.
Le H, Tfelt-Hansen P, Skytthe A, Kyvik KO, Olesen J. Increase in self-reported migraine prevalence in the Danish adult population: a prospective longitudinal population-based study. *BMJ Open* 2012; 2(4).

Twin studies have shown that the heritability of the migraine phenotype is 34–65%. This indicates that approximately 50% of the risk should be accounted for by environmental factors. Only few studies have addressed the association of environmental factors with migraine. Diseases that have so far been verified in large-scale population-based studies with a consistent positive association with migraine are depression, anxiety disorders, epilepsy, allergies and asthma. Regarding environmental factors, some large-scale studies found migraine to be associated with low socioeconomic status. Many environmental factors and diseases have not yet been explored in large-scale population-based studies or have received little attention. Thus, our know ledge of potential risk factors is limited. The purpose of the present project was, therefore, to identify environmental risk factors including co-morbid diseases associated with migraine.

The study population was based on t win cohorts born between 1931 and 1982 who were enrolled in the Danish Twin Registry (DTR), the oldest and most complete national twin registry in the world. The twins were representative of the whole Danish population with regard to migraine, diabetes, asthma and mortality. The present project is based on two omnibus questionnaire surveys conducted by the DTR in 1994 and 2002. Both questionnaires

included the same two migraine questions which have previously been validated. The data from bot h surveys was the basis for the prevalence and incidence study, cross-sectional studies, the longitudinal study and the co-twin control study in the present project. The "Twin Omnibus 1994" comprised 28,57 1 twin individual s aged 12–4 1 and the "Twin Omnibus 2002" 31,865 twin individuals aged 20–71. Lifetime prevalence of migraine for age 20 to 41 increased from 1994 to 2002 (18.5% vs. 24.5%) by 32.2% (95% CI: 27.0 to 37.3%; $p < 0.001$). The difference was primarily seen in the population older than 32 years. The increase was especially evident in migraine with aura (MA) (5.6% vs. 9.4%, $p < 0.001$), but also a significant increase in migraine without aura (MO) was found (13.0% vs. 15.1%, $p < 0.001$). In total, 21 conditions were co-morbid with lifetime migraine, MA 23 and MO 12. These conditions included cardiovascular disorders, musculoskeletal disorders, autoimmune rheumatic disorders, thyroid disorders, audio-vestibular disorders, atopic disorders, epilepsy and kidney stone. Co-morbidity of migraine and arterial hypertension treated with prescription drugs, kidney stone, osteoarthritis, low back pain, neck pain, whiplash and tinnitus were replicated in the co-twin control study. In the longitudinal study, low back pain was found to increase the risk of development of migraine. Increased risk of migraine was also found in subjects with low school and educational level; unemployed, homemakers and retired subjects; heavy physical workload; underweight and obese subjects; smokers and married subjects. Decreased risk of migraine was found in subjects with frequent alcohol consumption, heavy physical recreational activity; students and self-employed subjects. Frequent alcohol consumption was the only environmental factor significantly associated with migraine in the co-twin study. The longitudinal study showed that the risk of migraine development was increased in subjects with low education, heavy physical workload, heavy physical recreational activity and underweight. Subjects with frequent alcohol consumption had a Lower risk of developing migraine compared to subjects with less frequent or no consumption of alcohol.

In conclusion, this project demonstrated a substantial increase in the lifetime prevalence of migraine in Denmark from 1994 to 2002. An investigation of co-morbidities and environmental factors which may explain this increase was done, but association with the environmental factors, which were established, was not strong enough to explain the increase.

Although this project was not able to establish strong associations between migraine and the environmental factors, we are getting one step closer. Further studies using the co-twin design can add to understanding the influence of environmental factors on migraine.

Dipak V Amrutkar

Calcitonin gene-related peptide (CGRP) uptake and release in rat dura mater, trigeminal ganglion and trigeminal nucleus caudalis.

A PhD thesis based on the following works:
Gupta S, Amrutkar DV, Mataji A, Salmasi H, Sheykhzade M, Messlinger K, Olesen J, Jansen-Olesen I, 'Evidence for CGRP uptake in rat dura mater encephali: Relevance to migraine' *Br J Pharmacol* 2010; 161: 1885–98
Amrutkar DV, Ploug KB, Olesen J, Jansen-Olesen I, 'Role for Voltage Gated Calcium Channels in Calcitonin Gene-Related Peptide Release in the Rat Trigeminovascular System'. *Neuroscience* 2011; 172: 510–517
Amrutkar DV, Ploug KB, Hay-Schmidt A, Porecca F, Olesen J, Jansen-Olesen I. 'Messenger- RNA expression of 5-HT1B, 5-HT1D and 5-HT1F receptors and their role in controlling the release of calcitonin gene-related peptide (CGRP) in the trigeminovascular system', *Pain* 2012; 153: 830–838

Migraine is neurovascular disease affecting over 10% of the world population. It is considered as a disease of the trigeminal vascular system. According to the dural neurogenic inflammation hypothesis of migraine an unknown stimulus activates the trigeminal nerve causing neurotransmitter release from its peripheral branches innervating dura mater subsequently initiating an inflammatory cascade which may sensitize dural nociceptors. Another consequence of trigeminal nerve activation is the transmission of pain information to the central nervous system via the central branches of these same trigeminal neurons. Thus inhibition of trigeminal neurotransmitter release blocks both peripheral neurogenic inflammation and central pain transmission mechanism. An important neurotransmitter involved in the above proposed mechanism is calcitonin gene-related petide (CGRP). Antagonists of CGRP and triptans which inhibit release of CGRP have been found effective in treatment of acute migraine attack. The aim of the PhD thesis was to study CGRP uptake and release in the trigeminovascular system. The PhD thesis is based on three studies showing (i) CGRP uptake in rat dura mater, (ii) the effect of voltage gated calcium channels and (iii) serotonin receptors (5-HT 1B,1D and 1F) on CGRP release in the trigeminovascular system (TVS).

Real time PCR was used to identify the mRNA expression of P/Q -, N-, L-, and T- type VGC channels and 5-HT1B, 5-HT1D and 5-HT1F receptors in TVS. Immunohistochemistry was used to anatomically locate the endogenous CGRP and to study the effect of capsaicin treatment on it. An *ex-vivo* method was used to study CGRP release in TVS as measured by ELISA. Evidence for CGRP uptake in rat dura mater encephali: Our studies showed

that CGRP is released by capsaicin and it was inhibited by capsazepine and in the absence of Ca2+. Successive capsaicin challenges depleted endogenous CGRP from dural nerve fibers and it was replenished with exogenous CGRP. CGRP uptake was not mediated by the CGRP receptor and it was blocked 7 by a CGRP antibody and was dependent on Ca2+. Immunohistochemical studies showed anatomical evidence of CGRP uptake.

Role for Voltage Gated Calcium Channels in Calcitonin Gene-Related Peptide Release:

Messenger RNA expression of P/Q -, N-, L-, and T- type voltage gated calcium channels were found in dura mater, TG and TNC. The VGC channel blockers had no effect on basal CGRP release but significantly inhibited KCl induced CGRP release in dura mater and TG. A cocktail of the four VGC channel blockers significantly decreased the KCl induced CGRP release in all three migraine relevant tissues of TVS. Furthermore, KCl induced CGRP release is dependent on extracellular calcium.

Messenger- RNA expression of 5-HT1B, 5-HT1D and 5-HT1F receptors and their role in controlling the release of calcitonin gene-related peptide (CGRP) in the trigeminovascular system:

Messenger-RNA of 5-HT1B, 5-HT1D and 5-HT1F receptors was abundantly expressed in TG and TNC. The anti migraine drug, sumatriptan significantly inhibited KCl induced CGRP release in TVS. The effect of sumatriptan was reversed by a 5-HT1B/1D receptor antagonist GR127935, in dura mater and TG. The selective agonist of the 5-HT1F receptor LY344964, was effective in inhibiting KCl induced CGRP release from peripheral terminals while the 5-HT1D receptor agonist, PNU142633 was an effective inhibitor of CGRP release from central terminals of TVS.

In conclusion, we have shown functional evidence of CGRP uptake in dura mater which was not mediated by CGRP receptors but blocked by a CGRP antibody. Capsaicin and KCl induced CGRP release is governed by extracellular calcium. Agatoxin, conotoxin, and nimodipine blockers of P/Q- N- and L- type VGC channels, respectively blocked the KCl induced CGRP release significantly in dura mater. The selective 5-HT1F receptor agonist, LY344864 and 5-HT1D receptor agonist PNU142633 were effective in inhibiting KCl induced CGRP release from peripheral and central trigeminovascular terminals respectively. The findings from these studies has further documented 8 the existence of CGRP uptake in peripheral trigeminovascular nerve fibers and has shown the role of specific voltage gated calcium channels in KCl induced CGRP release. Finally the role of individual 5-HT receptor subtypes in KCl induced CGRP release has been shown. This will help to develop better and safer treatment for migraine prophylaxis and acute migraine attacks.

Deepak Kumar Bhatt

The role of vasodilatation in migraine pathophysiology. In vitro and in vivo studies in rat

A PhD thesis based on the following works:

Bhatt DK, Ploug KB, Ramachandran R, Olesen J, Gupta S. Activation of PAR-2 Elicits NO-Dependent and CGRP Independent Dilation of the Dural Artery. *Headache* 2010; 50: 1017–1030.

Bhatt DK, Gupta S, Jansen-Olesen I, Andrews JS, Olesen, J. NXN-188, a selective nNOS inhibitor and a 5-HT1B/1D receptor agonist, inhibits CGRP release in preclinical migraine models. *Cephalalgia* 2013; 33: 87–100

Bhatt DK, Gupta S, Olesen J, Jansen-Olesen, I. PACAP-38 infusion causes sustained vasodilation of the middle meningeal artery in the rat: Possible involvement of mast cells. *Cephalalgia*, 2014; 34, 877–886.

Bhatt DK, Gupta S, Ploug KB, Jansen-Olesen I, Olesen J. mRNA distribution of CGRP and its receptor components in the trigeminovascular system and other pain related structures in rat brain, and effect of intracerebroventricular administration of CGRP on Fos expression in the TNC. *Neuroscience Lett* 2014; 559: 99–104

Migraine is a severe and debilitating neurovascular disorder, effecting more than 15% of world population. Although triptans are the main specific antimigraine agents, there is still a significant proportion of the migraineurs who are non-responders to triptans. Recent failure of phase-3 clinical trial of a calcitonin gene-related peptide (CGRP) receptor antagonist, telcagepant, necessitates the development of new therapeutics for migraine treatment. There is an unmet need to come up with new validated preclinical migraine models and drug targets, so that more companies show interest in antimigraine drug development. This thesis is an effort to address new receptor systems and preclinical models, which can be used for preclinical migraine research. The dissertation is based on four studies.

Study 1: This study shows that protease-activated receptor-2 (PAR-2) activating peptides SLIGRLNH2 and 2-Furoyl-LIGRLO-NH2 were able to cause dilatation of meningeal blood vessels. The vascular responses of SLIGRL-NH2 are partially mediated by nitric oxide (NO) but are independent of CGRP as well as of mast cells. This study is first to demonstrate the presence of PAR-2 mRNA as well as protein throughout the trigeminal vascular circuit, pivotal to migraine pathogenesis.

Study 2: NXN-188, a putative anti-migraine drug, inhibits KCl-, capsaicin- and RTX-induced iCGRP release in migraine relevant cephalic tissues. NXN-188 also blocked capsaicin- and electrical stimulation-induced increase in middle meningeal artery diameter. The combination of

NXN-413 (a selective nNOS inhibitor) and sumatriptan (5-HT1B/1D agonist) inhibited simulated iCGRP release from skull halves and inhibited capsaicin-induced increase in middle meningeal artery diameter more than achieved by each compound alone. The 5-HT1B/1Dreceptor antagonist, GR127935, reversed the effect of sumatriptan but did not reverse the effect of NXN-188. Thus, the effect of NXN-188 was partially dependent on its nNOS inhibitory property and was independent of its 5-HT1B/1D receptor agonistic property.

Study 3: The long lasting flushing observed in parallel with a migraine-like headache after pituitary adenylate-cyclase activating peptide-38 (PACAP-38) infusion in humans aimed us to study if PACAP-38-induced vasodilation of MMA could be mediated via degranulation of MCs. We hypothesized that in the MCD rats the vascular responses to PACAP-38 will be less than in control rats due to a lack of vasodilatory products released during mast cell degranulation. The MMA dilatory responses to prolonged PACAP-38 infusions were indeed attenuated in MCD rats, indicating a role of MC degranulation in PACAP-induced dilatation of MMA.

Study 4: The mRNA for CGRP receptor components were widely distributed in the trigeminovascular pathway and different pain processing structures. A very low amount of CGRP gene, Calca was also detected in dura mater, hypophysis, TNC, hypothalamus, PAG, hippocampus and in cingulate cortex. After 30 min of CGRP infusion an activation of p-ERK in TNC, and activation of p-ERK, p-CREB and p-ATF-1 in dura mater was observed. CGRP did not activate Fos significantly in TNC at the mRNA and at the protein level. Zif268 protein expression was also not increased significantly. Infusion of CGRP in rats did not change the mRNA expression pattern of CGRP or its receptor components in TNC. Intracerebroventricular injection of CGRP did not increase Fos in TNC but gave a distinct pattern of Fos expression on outer covering of brain stem, made of pia mater and glia limitans, compared to SIF injected rats. In summary, our results suggest the involvement of PAR-2 in dilating dural arteries, which can be used in future for screening of possible antimigraine drugs i.e. PAR-2 antagonist or drugs targeting intracellular pathways activated by PAR-2 activation. The existing iCGRP release model and closed-cranial window model were used to identify mechanism of dual acting drug, NXN-188.

NXN-188 effectively blocked the release of iCGRP from dura mater, TG and TNC. PACAP-38-induced long lasting vasodilation was dependent on mast cell activation. In future, drug targeting mast cell stabilization or inhibiting the response of mast cell mediators can be tested in this model.

Although CGRP can activate p-ERK and p-CREB immediately after infusion it did not significantly activate neuronal activation markers Fos and Zif268 in TNC. This study suggest the requirement of a sensitize rat model to further improve the infusion model based on CGRP.

Dorte Kjeldgaard

Personality profile and psychological treatment of patients with chronic post-traumatic headache

A PhD thesis based on the following works:
Kjeldgaard D, Forchhammer HB, Teasdale TW, Jensen RH. Chronic posttraumatic headache after mild head injury: A descriptive study. *Cephalalgia* 2014; 34: 191–200.
Kjeldgaard, D., Forchhammer, H.B., Teasdale, T.W. and Jensen, R.H "Cognitive behavioral treatment for the chronic post-traumatic headache patient. *J Headache Pain*. 2014; 15:81

Chronic post-traumatic headache (CPTH) attributed to mild head injury is still a significant mystery for patients as well as for headache experts and is very costly for the society. CPTH is defined as a secondary headache in the International Classification of Headache Disorders (ICHD-2). The underlying mechanisms are unknown and management is complicated. Besides the biological aetiology, psychological factors have been suggested as both causing, triggering and maintaining factors of CPTH. Both pharmacological treatments as well as a psychological intervention have not yet shown promising results.

The aims of this thesis were to describe a larger CPTH population and, in detail, to compare this group with other chronic headache patients in terms of demographic, headache characteristics, medication, psychological distress and general personality characteristics.

For the CPTH patients the aim were also to develop, implement and evaluate a group intervention based on Cognitive behavioural therapy (CBT), in order to provide them with knowledge and strategies to manage their CPTH.

Ninety patients with CPTH and forty-five patients with other chronic headaches were enrolled from the Danish Headache Center study. All patients were interviewed about demographic and headache data. They completed The Harvard Trauma Questionnaire (HTQ), Rivermead Post Concussion Symptoms Questionnaire, SCL-90-R measuring affective distress, NEO-PI-R personality profile, quality of life SF-36 and a diagnostic headache diary.

The ninety patients with CPTH were by randomisation allocated to either the developed Cognitive Behavioural Therapy program or to a six-month waiting-list group. The effect parameters of the treatment were the results from some of the above mentioned questionnaires.

The main findings of our study were that the CPTH group had significantly less affiliation to the labour market compared to the matched headache control group whereas there were no differences on most of the demographic characteristic and the two groups were equally burdened by headache. Despite these similarities, the CPTH group experienced significantly more cognitive and somatic symptoms than the control group, but at the same time reported the same level of emotional symptoms. The CPTH group also rated their self-perceived health as more affected in terms of physical health, whereas psychological health was very similar in the two groups with the exception of social function.

Almost a third of our CPTH patients also had a score equal to or above the cut-off score for having a PTSD according to the HTQ, indicating a high level of psychological stress and possibly a poorer prognosis. No differences were found in reported level of psychological distress and CPTH patients were not characterised by a specific personality profile compared to other chronic headache patients. Among the CPTH patients having more than two years since trauma/with headache seemed overall to increase their disability.

The developed CBT intervention seems only to have a minor impact on the CPTH patients' quality of life, psychological distress and their headache, as measured by the instruments employed.

Anders Hougaard

Investigations of functional and structural changes in migraine with aura by magnetic resonance imaging

A PhD thesis based on the following works:
Hougaard A, Jensen BH, Amin FM, Rostrup E, Hoffmann MB, Ashina M. Cerebral Asymmetry of fMRI-BOLD Responses to Visual Stimulation. *PLoS One* 2015; 10: e0126477
Hougaard A, Amin FM, Hoffmann MB, Rostrup E, Larsson HBW, Asghar MS, et al. Interhemispheric differences of fMRI responses to visual stimuli in patients with side-fixed migraine aura. *Hum Brain Mapp.* 2014; 35: 2714–2723
Hougaard A, Amin FM, Hoffmann MB, Larsson HBW, Magon S, Sprenger T, et al. Structural gray matter abnormalities in migraine relate to headache lateralization, but not aura. *Cephalalgia* 2015; 35: 3–9.

Migraine sufferers with aura often report visual discomfort outside of attacks and many consider bright or flickering light an attack-precipitating factor. The nature of this visual hypersensitivity and its relation to the underlying pathophysiology of the migraine aura is unknown. A useful technology to study these features of migraine with aura (MA) is functional magnetic resonance imaging (fMRI), which has the potential not only to detect, but also to localize hypersensitive cortex. The main objective of this thesis was to investigate the cortical responsivity of patients with MA during visual stimulation using fMRI. To optimize sensitivity, we applied a within-patient design by assessing functional interhemispheric differences in patients consistently experiencing visual aura in the same visual hemifield.

To validate our data analysis methods, we initially studied healthy volunteers using single hemi-field visual stimulation and compared the "stimulated" hemispheres (i.e. hemispheres contralateral to the visual stimulation) to the "non-stimulated" hemispheres. We then applied this validated method of interhemispheric comparison of fMRI-blood oxygenation level dependent (BOLD) activation to compare left versus right hemisphere responses to symmetric full-field visual stimulation in 54 healthy subjects (study I). This study concluded that, a) the applied visual stimulation is effective in activating large expanses of visual cortex, b) interhemispheric differences in fMRI-BOLD activation can be determined using the proposed method, and c) visual responses to symmetric full-field visual stimulation are asymmetrically distributed between the cerebral hemispheres. We investigated the effects of migraine aura, by including 20 patients with frequent side-fixed visual aura attacks, i.e. >=90% of auras occurring in the same visual hemifield (study II). To circumvent bias relating to differences between right and left hemispheres (e.g. caused by physiological left/right bias, asymmetry of the visual stimulation or magnetic field inhomogeneity of the scanner), we included an equal number of patients with right- and left-sided symptoms. Further, we included 20 individually matched healthy controls with no history (including family history) of migraine. We compared the fMRI-BOLD responses to visual stimulation between symptomatic and asymptomatic hemispheres during the interictal phase and between migraine patients and controls. BOLD responses were selectively increased in the in the symptomatic hemispheres and localized in the inferior parietal lobule, the inferior frontal gyrus and the superior parietal lobule. The affected cortical areas comprise a visually driven functional network involved in oculomotor control, guidance of movement, motion perception, visual attention, and visual spatial memory. The patients also had significantly increased response in the same cortical areas when compared to controls.

Since these findings theoretically could depend on aura-related differences in brain structure, we performed additional analyses (study III) to determine the relation between migraine aura and structural, cortical and subcortical, grey matter abnormalities. We analyzed structural MRI data from the same 20 patients and applied voxel-based morphometry and surface-based morphometry on a whole-hemisphere level and for specific anatomical regions of interest.

Within-subject comparisons were made with regard to aura symptoms (N = 20 vs 20) and with regard to headache (N = 13 vs 13). We found no differences in grey matter structure with regard to aura symptoms in MA patients. Comparing the typical migraine headache side of the patients to the contralateral side revealed a difference in cortical thickness in the inferior frontal gyrus, which correlated significantly with the migraine attack frequency.

In conclusion, we validated a method of interhemispheric comparison of fMRI-BOLD responses to visual stimulation. By using this method we discovered a lateralized alteration of a visually driven functional network in patients with side-fixed aura. These findings suggest a hyperexcitability of the visual system in the interictal phase of migraine with visual aura. Further, this abnormal function is not dependent on lateralized abnormalities of gray matter structure. However, alteration of the inferior frontal cortex related to headache lateralization could indicate structural reorganization of pain inhibitory circuits in response to the repeated intense nociceptive input due to the headache attacks.

Christina Kruse

Role of phosphodiesterase 5 and cGMP signalling in cerebral arteries, cerebral blood flow, and headache -contributions to understanding migraine pathophysiology

A doctoral thesis in medical science based on the following works:

Kruuse C, Thomsen LL, Jacobsen TB, Olesen J. The phosphodiesterase 5 inhibitor sildenafil has no effect on cerebral blood flow or blood velocity, but nevertheless induces headache in healthy subjects. *J Cereb Blood Flow Metab* 2002; 22: 1124–1131

Kruuse C, Thomsen LL, Birk S, Olesen J. Migraine can be induced by sildenafil without changes in middle cerebral artery diameter. *Brain* 2003; 126: 241–247

Kruuse C, Frandsen E, Schifter S, Thomsen LL, Birk S, Olesen J. Plasma levels of cAMP, cGMP and CGRP in sildenafil-induced headache. *Cephalalgia* 2004; 24: 547–553

Kruuse C, Khurana TS, Rybalkin SD, Birk S, Engel U, Edvinsson L, Olesen J. Phosphodiesterase 5 and effects of sildenafil on cerebral arteries of man and guinea pig. *Eur J Pharmacol* 2005; 521: 105–114

Kruuse C, Hansen AE, Larsson HB, Lauritzen M, Rostrup E. Cerebral haemodynamic response or excitability is not affected by sildenafil. *J Cereb Blood Flow Metab* 2009; 29; 830–839

Kruuse C, Gupta S, Nilsson E, Kruse LS, Edvinsson L. Differential vasoactive effects of sildenafil and tadalafil on cerebral arteries. *Eur J Pharmacology* 2012; 674:345–51

Migraine is an episodic headache disorder affecting 16% of the population, possibly originating from aberrant neurovascular signalling. The pathophysiology of migraine is thought to involve altered response to calcitonin gene-related peptide (CGRP), serotonin (5-HT), cyclic guanosine monophosphate (cGMP) and/or nitric oxide (NO). It has been proposed that migraine pain is initiated by cerebral artery dilatation, increased peripheral nociception, or via a CNS based mechanisms. The effects of CGRP, 5-HT and NO are thought to be mediated by second messenger molecules cyclic adenosine monophosphate (cAMP) or cGMP. The overall aim of the studies was to investigate the role of cGMP signalling in the pain pathway of migraine by characterizing the distribution and activity of the specific cGMP degrading enzyme, phosphodiesterase 5 (PDE5) in the cerebral arteries by *in vitro* and *in vivo* methods. Further, the physiology of human headache was studied by using a model, in which healthy subjects and migraine patients were exposed to an inhibitor of PDE5, sildenafil, which increases cGMP levels and thus potentiates the NO signalling pathway.

Our data showed that sildenafil did induce headache in healthy subjects and migraine-like headache in migraine patients. However, sildenafil does not dilate cerebral arteries, change cerebral blood flow, or alter cerebrovascular reactivity to light or CO_2. In migraine patients, the sildenafil induced headache was monophasic and reached a maximal migraine-like intensity more rapidly than the GTN-induced headache, which is biphasic in nature. Sildenafil did not increase plasma CGRP, increase sensitivity to peripheral pain or amplitude of visual-evoked responses. These findings are the first to show an induction of headache in the absence of cerebral artery dilatation. In vitro studies accordingly showed that sildenafil dilated isolated human and rodent cerebral arteries only at high concentration despite presence of active PDE5, and only when applied on the outside of the artery. Tadalafil, another selective PDE5 inhibitor, did not dilate cerebral arteries even at high concentrations, though it is reported to induce headache. Although sildenafil may have effects on rat dural arteries *in vivo,* the dilatation is accompanied by a decrease in blood pressure not seen in humans. In summary, the data presented here show that sildenafil induces migraine-like symptoms in healthy subjects and migraine patients without increasing the diameter of cerebral or extra-cerebral arteries or other apparent vascular effects and it does not dilate cerebral arteries *in vitro*. These data support the idea that vasodilatation previously reported in migraine may be an epiphenomenon to the headache and that migraine pain pathway is independent of vascular dysfunction. Future studies should address the possible neuronal effects of sildenafil as well as the possible interplay between cGMP, CGRP and serotonin in the context of the

migraine pain pathway. PDE5 activation could be e new target in migraine treatment.

Hanne Maria Yri

Idiopathic intracranial hypertension – Exploring headache and cognitive function

A PhD thesis based on the following works:
Yri HM, Jensen RH. Idiopathic intracranial hypertension:clinical nosography and field-testing of the ICHD diagnostic criteria – a case-control study. *Cephalalgia* 2015; 35: 553–562.
Yri HM, Fagerlund B, Forchhammer HB, Jensen RH. Cognitive function in idiopathic intracranial hypertension: a prospective case-control study. *BMJ Open* 2014; 4(4).

Idiopathic intracranial hypertension is a condition characterized by raised intracranial pressure (ICP) of unknown cause predominantly seen in young obese women. Although still relatively infrequent the disorder has a substantial socioeconomic impact, prone to increase as the global obesity epidemics causes the prevalence to rise. Major expenses are caused by patients having to give up work or change profession due to IIH [38] and studies show significant reduction of life quality [39]. Although patient in addition often report difficulties in memory and concentration the impact of IIH on cognitive function has only been sparsely and inconsistently described in the literature.

The overall objective of this PhD-project was to explore the clinical presentation, and the headache in IIH. We aimed to identify characteristics distinguishing IIH headache from other headache disorders in attempts to improve the present diagnostic criteria; to describe the course of headache during the first year after diagnosis and to explore mechanisms of headache chronification. In addition we aimed to explore the extent and nature of cognitive deficits in IIH in a controlled design.

We investigated headache by diaries kept by the patients for the three first months of diagnosis and by standardized semi-structured interviews performed at every visit. The immediate effect of ICP normalisation was tested by registration of headache before and after CSF withdrawal and pain perception was explored by Quantitative sensory testing. Comparisons of headache patients with and without IIH showed that headache attributed to IIH was more likely to aggravate by coughing or straining, to be retrobulbar and to improve after CSF withdrawal compared to headache in patients with normal ICP. Based on these and other finding the sensitivity and specificity of the present diagnostic criteria of the International Classification of Headache Disorders could be improved from 86% – 95% and from 53% – 65% respectively. In addition the clinical applicability of the criteria could be improved.

The most dramatic improvement in headache occurred within the first weeks of diagnosis. After one month headache has resolved completely in 26% and was reduced by more than 75% in another 40%. After one year 43% reported headache $\leq$2 days/months. However, in the majority of the remaining patients (43% of all patients) headache persisted as a chronic symptom although more than half of them had no history of pre-existing headache. Early age of onset and high diagnostic pressure were associated with better headache outcome indicating that mechanisms may differ with age. In general visual outcome after one year was very good.

In spite of high frequency of headache chronification, investigation of pain perception in patients with IIH provided no evidence of central sensitization either at diagnosis or after three months.

Cognitive function was tested by a comprehensive battery of validated neuro-psychological paper-and-pencil tests and computerized tests from the Cambridge Neuropsychological Test Automated battery. We demonstrated that patients with IIH performed significantly worse in four of the six tested domains. The most pronounced deficits were found in the domains of reaction time and processing speed. Despite substantial improvement in headache and normalisation of ICP in half of the patients cognitive deficits persisted after three months of treatment suggesting that IIH is accompanied by generalised cognitive impairment insufficiently treated by standard ICP regulating therapy that might contribute to reduced work capacity in patients with IIH.

In conclusion this project supported the clinical impression of marked cognitive impairment in patients with IIH which in addition to the high rate of headache chronification may explain some of the socioeconomic disability found in patients with IIH. The failure of standard treatment to alleviate headache in almost half of the patients stresses the importance of investigations of headache mechanisms in IIH. Central sensitization has been shown to play a key role in other chronic headache disorders but QST performed in this project provided no evidence of this mechanism in IIH.

In conclusion we found that sustained cognitive dysfunction and chronic headache by far exceeds the extent of visual complications in patients treated with diuretics according to international guidelines. The question of mechanisms underlying these complications remains unsolved and further studies are needed to provide basis for improved headache therapy and treatment of cognitive deficits.

Henrik Schytz

Near infrared spectroscopy investigations in neurovascular diseases

A doctoral thesis in medical science based on the following works:
Schytz HW, Wienecke T, Jensen LT, Selb J, Boas DA & Ashina M. Changes in cerebral blood flow after acetazolamide: an experimental study comparing near-infrared spectroscopy and SPECT. *Eur J Neurol* 2009; 16: 461–467.
Phillip D, Schytz HW, Selb J, Payne S, Iversen HK, Skovgaard LT, Boas DA & Ashina M. Low frequency oscillations in cephalic vessels assessed by near infrared spectroscopy. *EurJ Clin Inves* 2012; 42: 1180–1188.
Schytz HW, Ciftçi K, Akin A, Ashina M & Bolay H. Intact neurovascular coupling during executive function in migraine without aura: interictal near-infrared spectroscopy study. *Cephalalgia* 2010; 30: 457–466.
Schytz HW, Hansen JM, Phillip D, Selb J, Boas DA & Ashina M. Nitric oxide modulation of low-frequency oscillations in cortical vessels in FHM – a NIRS study. *Headache* 2012; 52: 1146–1154.
Schytz HW, Barløse M, Guo S, Selb J, Caparso A, Jensen R & Ashina M. Experimental activation of the sphenopalatine ganglion provokes cluster-like attacks in humans. *Cephalalgia* 2013; 33: 831–841.
Schytz HW, Jensen BE, Jennum P, Selb J, Boas DA & Ashina M. Low-frequency oscillations and vasoreactivity of cortical vessels in obstructive sleep apnea during wakefulness: A near infrared spectroscopy study. *Sleep Medicine* 2013; 14: 416–421.
Schytz HW, Guo S, Jensen LT, Kamar M, Nini A, Gress DR & Ashina M. A new technology for detecting cerebral blood flow: a comparative study of ultrasound tagged NIRS and 133Xe-SPECT. *Neurocritical Care* 2012; 17: 139–145.

The purpose of this thesis was to explore and develop methods, where continuous wave near infrared spectroscopy (CW-NIRS) can be applied in different neurovascular diseases, in order to find biological markers that are useful in clinical neurology. To develop a new method to detect changes in cerebral blood flow (CBF), the first study investigated a multi-source detector separation configuration and indocyanine green (ICG) as a tracer to calculate a corrected blood flow index (BFI) value. The study showed no correlation between CBF changes measured by 133Xenon single photon emission computer tomography (^{133}Xe-SPECT) and the corrected BFI value. It was concluded, that it was not possible to obtain reliable BFI data with the ICG CW-NIRS method. NIRS measurements of low frequency oscillations (LFOs) may be a reliable method to investigate vascular alterations in neurovascular diseases, but this requires an acceptable LFOs variation between hemispheres and over time in the healthy brain. The second study therefore investigated day-to-day and hemispheric variations in LFOs with NIRS. It was shown that NIRS might be useful in assessing LFOs between hemispheres, as well as interhemispheric phase and gain directly and over time. Migraine may be associated with persistent impairment of neurovascular coupling, but there is no experimental evidence to support this. The third study therefore investigated interictal neurovascular coupling during a mental task by a Stroop test in migraine without aura (MO) patients, which is the most common type of migraine. The study showed intact neurovascular coupling in the prefrontal cortex outside of attacks in patients with MO. The fourth study aimed to investigate possible changes in LFOs amplitude following nitric oxide (NO) donor infusion in familial hemiplegic migraine (FHM), which is a rare Mendelian subtype of migraine with aura. This study showed increased LFOs amplitude only in FHM patients with co-existing common type of migraine, but not in patients with pure FHM phenotype. This suggests that the sensitivity to NO resides within the common migraine phenotypes rather than the FHM phenotype. Stimulation of the sphenopalatine ganglion (SPG) may lead to parasympathetic outflow and cause pain in cluster headache (CH). The fifth study therefore investigated pain and autonomic symptoms in relation to high or low SPG frequency stimulation in chronic CH patients. Cortical changes in oxygenated hemoglobin (HbO) were also recorded with NIRS and showed a moderate HbO increase, which was most pronounced on the ipsilateral CH side following high frequency stimulation. A possible application of NIRS to assess cerebral vascular changes due to sympathetic activity was investigated in obstructive sleep apnea (OSA) patients, who have increased sympathetic activity and risk of stroke. Following successful continuous positive airway pressure (CPAP) therapy, OSA patients decreased their LFOs amplitude, which was interpreted as a marker of decreased sympathetic activity in cortical vessels. Finally, a novel hybrid technique, combining NIRS and ultrasound, was tested to detect CBF changes after acetazolamide injection in healthy volunteers using a cerebral flow index (CFI). The study showed an increase in CFI, which correlated with CBF measured with ^{133}Xe-SPECT at 15 min, but not 60 min. Further methodological and explorative clinical studies are needed to assess the feasibility of ultrasound tagged NIRS in clinical neurology. In summary, the thesis presents several novel approaches, by which NIRS may be used in clinical neurology, and potentials of NIRS to investigate complex mechanisms in neurovascular diseases.

Faisal Amin

Clinical and physiological characterization of PACAP38-induces headache and migraine

A PhD thesis based on the following works:
Amin FM, Lundholm E, Hougaard A, et al. Measurement precision and biological variation of cranial arteries using automated analysis of 3 T magnetic resonance angiography. *J Headache Pain.* 2014; 15: 25.
Amin FM, Asghar MS, Ravneberg JW, et al. The effect of sumatriptan on cephalic arteries: A 3T MR-angiography study in healthy volunteers. *Cephalalgia.* 2013; 33: 1009–1016.
Amin FM, Asghar MS, Guo S, et al. Headache and prolonged dilatation of the middle meningeal artery by PACAP38 in healthy volunteers. *Cephalalgia*. 2012; 32: 140–149.
Amin FM, Hougaard A, Schytz HW, et al. Investigation of the pathophysiological mechanisms of migraine attacks induced by pituitary adenylate cyclase-activating polypeptide-38. *Brain.* 2014; 137: 779–794.

The overall purpose of the present thesis was to study the role of the pituitary adenylate cyclase-activating polypeptide-38 (PACAP38) in relation to migraine without aura. PACAP38 is a potent vasodilator and appears to play an important role in the pathophysiology of migraine without aura. We investigated vascular PACAP38 mechanisms in migraine without aura using high-resolution magnetic resonance angiography (MRA) in the present work. In the first study, we tested the measurement precision of the MRA analysis method and the biological (day-to-day and side-to-side) variations of cranial arteries. This study revealed excellent reproducibility of the MRA analysis method and showed that both day-to-day and side-to-side variations of cranial arteries were below 10%. To further explore the MRA method in order to detect arterial circumference changes the selective antimigraine drug sumatriptan (a 5-$HT_{1B/1D}$ receptor agonist) was used in the second study. This study showed marked circumference reductions of the smaller extracerebral arteries but not the larger cerebral arteries. Sumatriptan receptors have been shown in the human extracerebral as well as cerebral arteries and previous *in vitro* studies have shown comparable smooth muscle constriction effects of sumatriptan in both types of arteries. Thus, the extracerebral selectivity of the constrictor effect of sumatriptan was suggested to be caused by its inability to cross the blood-brain barrier to a high degree. In the third study, the immediate and delayed effects of intravenous PACAP38 infusion on cranial arteries were investigated in healthy subjects. PACAP38 caused huge and prolonged dilatation of the middle meningeal artery (MMA) but not the middle cerebral artery (MCA). All participants experienced as minimum a very mild headache during the experiment. PACAP38-induced dilatation and headache were both reversed by sumatriptan in this study. Eventually, the vascular migraine mechanisms of PACAP38 were investigated in 24 migraine without aura patients in the forth study. This study used a double-blind randomized crossover head-to-head comparison design to compare PACAP38 and the vasoactive intestinal polypeptide (VIP). Both peptides are structurally closely related and act via the three shared VPAC1, VPAC2 and PAC1 receptors. While both PACAP38 and VIP bind with equal affinity to the two first, PACAP38 has more 100 times higher affinity for the PAC1 receptor than VIP. The study confirmed that PACAP38 caused significantly more migraine attacks than VIP in patients (73% versus 18%). However, both peptides caused marked extracranial but no intracranial arterial dilatation. PACAP38-induced dilatation lasted for more than 5 hours, whereas the arterial circumferences were normalized after 2 hours on the VIP day. In addition, blood samples revealed that the plasma concentration of PACAP38 was unexpectedly high 1 hour after infusion start of PACAP38. Explorative analyses showed that plasma PACAP38 was higher in patients who later developed migraine attacks compared to those who did not experienced PACAP38-induced migraine attacks. MRA was done in nine patients with PACAP38-induced unilateral migraine attacks. These scans showed marked extracranial but no intracranial arterial dilatations. However, there were no side-to-side differences in the extracranial arteries. The same extracranial arteries were also dilated in patients who had no attacks. Thus, extracranial dilatations were ascribed a PACAP38 effect rather than caused by migraine effect. Sumatriptan reversed both migraine attacks and extracranial dilatations. The vasodilator effect of PACAP38 and vasoconstrictor effect of sumatriptan was almost the same in patients (study IV) and healthy controls (study II and III). It was concluded that PACAP38-induced vasodilation per se, may not be a relevant mechanism for migraine induction as well as extracranial vasodilatation is not a biomarker of PACAP38-induced migraine attacks. However, these studies could suggest that the underlying mechanisms of PACAP38-induced migraine may include peripheral initiating mechanisms.

Mads Barloese

Sleep and neurobiology in cluster headache

A PhD thesis based on the following works:
Barloese M, Brinth L, Mehlsen J, et al. Blunted autonomic response in cluster headache patients. *Cephalalgia* 2015; 35; 1269–1277.
Barloese M, Jennum P, Knudsen S, et al. Cluster headache and sleep, is there a connection? A review. *Cephalalgia* 2012; 32: 481–491.
Barloese M, Jennum P, Lund N, et al. Reduced CSF hypocretin-1 levels are associated with cluster headache. *Cephalalgia* 2015; 35: 869–876.

Barloese M, Lund N, Petersen A, et al. Sleep and chronobiology in cluster headache. *Cephalalgia* 2015; 35: 969–978.
Barloese MC, Jennum PJ, Lund NT, et al. Sleep in cluster headache – beyond a temporal rapid eye movement relationship? *Eur J Neurol* 2015; 22: 656–e40.

Cluster headache is characterized by unilateral attacks of severe pain accompanied by cranial autonomic features. Apart from these there are also sleep-related complaints and strong chronobiological features. The interaction between sleep and headache is complex at any level and evidence suggests that it may be of critical importance in our understanding of primary headache disorders. In cluster headache several interactions between sleep and the severe pain attacks have already been proposed. Supported by endocrinological and radiological findings as well as the chronobiological features, predominant theories revolve around central pathology of the hypothalamus. We aimed to investigate the clinical presentation of chronobiological features, the presence of concurrent sleep disorders and the relationship with particular sleep phases or phenomena, the possible role of hypocretin as well as the possible involvement of cardiac autonomic control. We conducted a questionnaire survey on 275 cluster headache patients and 145 controls as well an in-patient sleep study including 40 CH-patients and 25 healthy controls. The findings include: A distinct circannual connection between cluster occurrence and the amount of daylight, substantially poorer sleep quality in patients compared to controls which was present not only inside the clusters but also outside, affected REM-sleep in patients without a particular temporal connection to nocturnal attacks, equal prevalence of sleep apnea in both patient and control groups, reduced levels of hypocretin-1 in the cerebrospinal fluid of patients and finally a blunted response to the change from supine to tilted position in the head-up tilt table test indicating a weakened sympathoexcitatory or stronger parasympathetic drive. Overall, these findings support a theory of involvement of dysregulation in hypothalamic and brainstem nuclei in cluster headache pathology. Further, it is made plausible that the headache attacks are but one aspect of a more complex syndrome of central dysregulation manifesting as sleep-related complaints, sub-clinical autonomic dysregulation and of course the severe attacks of unilateral headache. Future endeavors should focus on pathological changes which persist in the attack-free periods but also heed the possibility of long-lived, cluster-induced pathology.

Ann-Louise Esserlind

Following the leads in migraine genetics – An investigation of migraine susceptibility loci

A PhD thesis based on the following works:
Esserlind AL, Kirchmann M, Hauge AW, Le H & Olesen J. A genotype-phenotype analysis of the 8q22.1 variant in migraine with aura. *Eur J Neurol* 2012; 19: 603–609.
Esserlind AL, Christensen AF, Le H, Kirchmann M, Hauge AW, Toyserkani NM, Hansen T, Grarup N, Werge T, Steinberg S, Bettella F, Stefanssón H; Olesen J. Replication and meta-analysis of common variants identifies a genome wide significant locus in migraine. *Eur J Neurol* 2013; 20: 765–772.
Esserlind AL, Christensen AF, Steinberg S, Grarup N, Pedersen O, Hansen T, Werge T, Hansen T, Husemoen LLN, Linneberg A, Budtz-Jorgensen E, Westergaard ML, Stefanssón H, Olesen J. The association between candidate migraine susceptibility loci and severe migraine phenotype in a clinical sample. *Cephalalgia* 2015; 36: 1–9.

The aim of the PhD thesis was to assess the single nucleotide polymorphisms (SNPs) found to be associated with migraine in large genome-wide association studies (GWAS), in an independent and clinic based migraine cohort. The focus was to investigate whether these migraine-associated SNPs were preferentially associated with specific migraine features. We carried out three studies, where we investigated the association of previously detected SNPs with migraine. The first study analyzed the risk allele of rs1835740 (*MTDH*/*PGCP*), which was found to be associated with migraine with aura in the original study by Verneri et al. 2010, but we did not find any association to specific aura features in our clinical sample. In the second study we followed up on findings from the subsequent GWAS by Chasman et al. 2013, where we replicated two of the three migraine-associated SNPs from the original study, namely rs10166942 (near *TRPM8*) and rs1117213 (*LRP1*). We then performed a meta-analysis by combining our data with that of the original publication, and found further evidence of association of rs2078371 (near *TSPAN2*) with migraine. We did not find an association of any of these SNPs with specific migraine traits in our clinical cohort. The lack of association is most likely due to lack of statistical power, i.e. small sample size. In the third study, we followed up on 12 migraine loci detected by a large GWAS meta-analysis by Verneri et al. 2013. We replicated the association with the *LRP1* and *TSPAN2* loci and in addition found that rs2274316 (*MEF2D*), rs9349379 (*PHACTR1*) and rs11759769 (*FHL5*) reach significant association with migraine in our meta-analysis. In line with the original report, the associated loci were associated with migraine without aura but not with migraine with aura. Further, we found that the *LRP1* and *MEF2D* loci are associated with increased frequency of migraine attacks, which

is the cardinal symptom of severity in our clinic-based migraine sample. Interestingly, both the *LRP1* and *MEF2D* loci are located close to genes involved in glutamate neurotransmission in the brain. Although speculative, these finding points toward a general hyperexcitability that may be the underlying cause for severely affected migraine sufferers with frequent migraine attacks. To conclude, in our follow-up studies we found five migraine loci to be associated with migraine and secondary analysis suggest that the putative genes affected have a plausible biologically function in the brain. Thus, we suggests that the next step should be to investigate the biological function of these loci.

Stine Maarbjerg

Classical trigeminal neuralgia – clinical characteristics and diagnostic criteria

A PhD thesis based on the following works:
Maarbjerg S, Gozalov A, Olesen J, Bendtsen L. Trigeminal neuralgia – a prospective systematic study of clinical characteristics in 158 patients. *Headache* 2014; 54: 1574–1582.
Maarbjerg S, Gozalov A, Olesen J, Bendtsen L: Concomitant persistent pain in classical trigeminal neuralgia – evidence for different subtypes. *Headache* 2014; 54: 1173–1183.
Maarbjerg S, Sørensen MT, Gozalov A, Bendtsen L, Olesen J: Field-testing of the ICHD-3 beta diagnostic criteria for classical trigeminal neuralgia. *Cephalagia* 2015; 35: 291–300.

Classical trigeminal neuralgia (TN) is a relatively rare neuropathic disease characterized by intense stabbing pain in one side of the face. There is a lack of studies describing the clinical characteristics based on a large population of TN patients representative of the full disease spectrum from mild to severe symptoms and from classical presentations to borderline cases.

The objective of this thesis was to describe the clinical characteristics in TN and especially in relation to TN with concomitant persistent pain to examine its prevalence, characteristics and possible associations to other clinical characteristics. Finally, it is an objective to field-test the current international diagnostic criteria of TN.

To meet these objectives a cross-sectional study design was employed. Data were collected systematically and prospectively by semi-structured interviews from consecutive TN patients attached to the Danish Headache Center.

The study describes clinical characteristics such as sex distribution, age of onset, pain characteristics, associated symptoms, response to medical treatment and findings at clinical examination. Concomitant persistent pain was present in half the patients and was found to be associated to the female sex and to sensory abnormalities at clinical examination. In contrast to previous theories, TN with concomitant persistent pain was not associated to duration of disease.

The thesis documents that the recently revised diagnostic criteria in TN are not significantly more sensitive or specific compared to the previous diagnostic criteria, and argues why a high sensitivity is crucial. This can be achieved by a modification allowing for sensory abnormalities at clinical examination.

Thus, the thesis sheds light on what characterizes TN, describes the phenotype TN with concomitant persistent pain in detail and suggests ways to improve the diagnostic criteria.

Maria Westergaard

Medication-overuse headache: burden, consequences, prevention

A PhD thesis based on the following works:
Westergaard ML, Hansen EH, Glurner C, Olesen J, Jensen RH. Definitions of medicationoveruse headache in population-based studies and their implications on prevalence estimates: A systematic review. *Cephalalgia* 2014; 34: 409–425.
Westergaard ML, Glümer C, Hansen EH, Jensen RH. Prevalence of chronic headache with and without medication overuse: Associations with socioeconomic position and physical and mental health status. *Pain* 2014; 155: 2005–2013.
Westergaard ML, Glümer C, Hansen EH, Jensen RH. Medication overuse, healthy lifestyle behaviours and stress in chronic headache: results from a representative population-based survey. *Cephalalgia* 2016; 36; 15–28.
Westergaard ML, Hansen EH, Glümer C, Jensen RH. Prescription pain medication for chronic headache in Denmark: implications for preventing overuse. *Eur J Clin Pharmacol* 20 15; 71: 851–860.

Medication-overuse headache (MOH) is a condition where medications that are expected to relieve headache instead contribute to its worsening. The purpose of this research project was to investigate the prevalence of MOH, to describe its consequences for the individual and for public hea lth, and to determine modifiable factors associated with MOH wh ich could be targets for public health intervention. The specific objectives of this research project were: (l) to review prevalence reports of MOH worldwide and the various case definitions used in population-based research on MOH; (2) to estimate the prevalence of MOH in Denmark; (3) to investigate the burden of MOH in terms of reduced quality of life and how this burden might be mediated by socioeconomic position; (4) to

analyse associations between MOH, unhealthy lifestyle behaviour, and stress; (5) to detennine which prescription pain medications are most commonly dispensed to people with chronic headache, and to describe the volume dispensed to those with MOH and those with chronic headache but no medication overuse (CHnoO).

A systematic literature review summarizing the results of 27 population-based studies on MOH showed that most prevalence estimates are between 1 to 2% among adults, with a range between 0.5% to 7.2% worldwide.

The prevalence of MOH in Denmark was previously not known. With data from 68,518 respondents from the Danish National Health Survey 2010, this project is so far the largest prevalence study on MOH. Prevalence was estimated at 1.8% (CI: 1.7–1.9%) after adjustments for stratified sampling and non -response. MOH was more prevalent among women, the middle aged, and people with non-Danish and non-western ethnicity. The groups with the highest prevalence were people on social welfare (11.0%), early pensioners (7.5%), and those receiving sickness benefits (6.0%).

The quality of li fe of people with MOH (as measured by the 12-item Medical Outcomes Study Short Form Survey, SF-12) was significantly lower than the average for the population, and tended to be lower than those wi th CHnoO. SF-12 scores were low in all socioeconomic categories, suggesting that high socioeconomic position does not necessarily "protect" against low quality of life in people with MOH, while those who start out with a low socioeconomic position will likely have fewer resources to cope in the long term.

People with higher stress (measured by the Perceived Stress Scale) had greater odds for both MOH and CHnoO. However, associations with daily smoking, physical inactivity, and obesity were significant for MOH but not for CHnoO. Odds for MOH were highest among people who had all these three harmful health behaviors compared to those who had none (OR 2.8 in women and 5.1 in men). High stress plus any of these three behaviors had synergistic effects in MOH but not clearly in those who had CHnoO. There was no statistically significant association between MOH and excessive drinking or illicit drug use (among young adults).

Most people with MOH overused over-the-counter (OTC) analgesics, but they also used prescription pain med ications to a greater extent than those with CHnoO (in terms of average amount dispensed per person in one year). Prescription registry data showed that among those with MOH, NSAIDs were dispensed to 36.6%, opioids 32.4%, other analgesics 26.9%, and triptans 6.2%. The medications most commonly dispensed were paracetamol, tramadol. ibuprofen and codeine. Average defined daily doses per person for these four medications were 11, 18, 4 and 8 times higher, respectively, for the MOH group compared to the group with CHnoO.

Opioid use among people with MOH suggests either inappropriate treatment of chronic headache with opioids or the development of MOH among t hose treated with opioids for other chronic pain. Although there were cases of triptan overuse, overall triptans seemed underutilized considering that an estimated one-third to one-half of people with MOH have migraine. In a dequate treatment of migraine could lead to headache chronification as well as overuse of inappropriate or less effective medications.

The high individual and public health burden of chronic headache can be reduced by preventing and treating MOH. Among demographi c groups with high prevalence of MOH, it is important to look at those who have high levels of perceived stress and those engaged in behaviors such as daily smoking, physical inactivity or unhealthy eating. Stress reduction and health promotion are highly relevant in MOH management. Education of medication consumers, patients with MOH, and health care providers regarding appropriate headache management is essential for the prevention of MOH.