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Pain, autonomic activation and muscular activity related to experimentally-induced cognitive stress in headache patients

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Preface and acknowledgements

I wish to express my sincere gratitude to a number of people who I have had the pleasure to work with over the last years: first of all I wish to thank my supervisors Trond Sand and Lars Jacob Stovner, for their brilliant and enthusiastic supervision. They patiently introduced me to the fields of headache, neurophysiology and statistics, and they have guided and encouraged me during my work here. I truly could not have asked for more. I also wish to thank my co-supervisor Linda White for her great supervision, and for accepting me as a diploma student back in 2003. Our discussions over countless cups of tea have meant a lot to me. Furthermore I wish to thank my co-author and office partner Kristian Bernhard Nilsen for his valuable comments and companionship, and co-author Rolf Westgaard for his major contributions to this field and to this thesis. I also thank my collegues Marit Stjern, Sylvia Nome Kvam and Grethe Helde for their contributions during the data collection.

I am most grateful to my mother Astrid, my brother Espen, and Finn-Erik, for their support over the years, and to my dear Nicola, for her love and encouragement. I could not have done this without you all.

This thesis is dedicated to my father. I wish he was still with us.

List of papers

Paper I   Leistad RB, Sand T, Westgaard RH, Nilsen KB, Stovner LJ. 2006. "Stress-induced pain and muscle activity in patients with migraine and tension-type headache." Cephalalgia 26(1): 64-73.

Paper II   Leistad RB, Sand T, Nilsen KB, Westgaard RH, Stovner LJ. "Cardiovascular responses to cognitive stress in patients with migraine and tension-type headache." Submitted.

Paper III   Leistad RB, Stovner LJ, White LR, Nilsen KB, Westgaard RH, Sand T. 2007. "Noradrenaline and cortisol changes to low-grade cognitive stress differ in migraine and tension-type headache." J Headache Pain 8:71-76.

Summary in English

Background and objectives

Stress is in several studies mentioned as the most frequent trigger of headache. Nevertheless, the exact mechanisms by which stress induces headache is poorly understood and relatively few studies with rigorous scientific methods mimicking real life settings have been performed. In the present study an experimental model was used to study muscular, cardiovascular and biochemical responses to cognitive low-grade stress and investigate relationships between these responses and pain in the head and shoulder/neck areas.

Methods

This thesis is based on data recorded during and after a stress test in healthy controls and patients with migraine or tension-type headache (TTH). The stress test consisted of a two-choice reaction time test designed to imitate real-life working conditions in a stressful office environment. The stress test lasted for 60 minutes and was followed by 30 minutes of relaxation. We recorded pain and surface electromyographic (EMG) activity in the trapezius, splenius, temporalis and frontalis muscles, in addition to blood pressure (BP), heart rate (HR), skin blood flow (BF) in the fingers as well as noradrenaline, adrenaline and cortisol levels in blood sampled before and after the stress test.

Results

The main findings were higher pain responses in the temporalis and frontalis areas (with similar trends for trapezius and splenius) and more potentiation of pain during the test when comparing TTH patients with controls. Migraine patients developed more pain in the splenius and temporalis areas than controls. TTH patients had a more generalised pain increase in all areas, while pain increase was more regional (more pain in the neck/shoulder compared to the head) in migraine. TTH patients had delayed pain recovery in all areas compared to controls, while migraine had delayed pain recovery in

the trapezius and temporalis areas. The temporalis EMG response was increased in migraineurs compared to controls, but there were no other differences in EMG responses between the diagnostic groups, and EMG responses were not correlated with pain responses. TTH patients had delayed EMG recovery in the trapezius compared to controls and migraineurs.

Cardiovascular responses to cognitive stress in migraine patients did not differ from those in control subjects. In TTH patients, a lack of HR-adaptation during stress was found and a trend towards a delayed systolic BP response during stress was also observed. Finger BF recovery was delayed after stress and stress-induced pain was associated with less vasoconstriction in TTH during recovery.

TTH patients had significantly less cortisol change to stress than controls and migraineurs, which has to our knowledge not been reported before. Migraineurs had lower noradrenaline levels in blood platelets compared to controls, and pain responses correlated negatively with noradrenaline levels in migraineurs.

Conclusions

Our results suggest that both patients with migraine and TTH respond differently to stress compared to controls. The increased pain responses and potentiation of pain observed in TTH support the hypothesis that central sensitization is important in these patients, and together with EMG recovery data suggest that TTH patients may be sensitized both in nociceptive and somatomotor neural pathways. Data on migraineurs points towards more limited regional pain sensitization and suggests that neck pain may be a trigger or prodrome to stress-induced migraine attacks in some patients. Surface-detectable muscular activity during stress did not appear to be causal for pain in migraine or TTH patients.

Based on pain, cardiovascular and cortisol data we hypothesize that TTH patients may have different stress adaptive mechanisms than controls and migraineurs, involving regulation of the cardiovascular system, the hypothalamus-pituitary-adrenocortical axis and pain control systems.

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For migraineurs, the lower noradrenaline levels and the inverse correlation found between noradrenaline and pain responses suggests that the sympathoneural response to stress appears to be closely linked with stress-induced pain in these patients.

Summary in Norwegian

Bakgrunn

Stress er i flere studier oppgitt som den vanligste utløsende faktor til hodepine, men mekanismene som forårsaker dette er ikke kjent. Få tidligere studier som har undersøkt sammenhenger mellom stress og hodepine har benyttet standardiserte modeller som samtidig kan relateres til stress i hverdagen. I denne studien ble muskulære, kardiovaskulære og biokjemiske responser til standardisert lav-gradig stress undersøkt og sammenlignet med smerteutvikling i skulder/nakke og hode.

Metoder

Denne avhandlingen baserer seg på data samlet inn fra friske kontroller og pasienter med migrene eller tensjonshodepine, under og etter en stress-test. Stress-testen besto av en 2-valgs reaksjons-tid test laget for å imitere arbeidsforhold i et stressende kontormiljø. Testen varte i 60 minutter og ble etterfulgt av 30 minutter med hvile. Det ble registrert data for utvikling av smerte og overflate elektromyografi i skulder, nakke, tinning og panne, blodtrykk, hjerterate, hud-blodstrøm i fingrene samt nivå av noradrenalin, adrenalin og kortisol i blodprøver tatt før og etter stress-testen.

Resultater

Tensjonshodepine-pasienter utviklet mer smerte i tinning og panne (og en lignende tendens i skulder/nakke) og en større grad av potensiering (økning) av smerte i løpet av testen sammenlignet med kontroller. Migrenepasienter utviklet mer smerte i nakke og tinning sammenlignet med kontroller. Tensjonshodepine hadde en mer generalisert smerteøkning i alle områder, mens for migrenepasienter var smerteøkningen mer regionalt sentrert rundt nakke/skulder. Tensjonshodepine-pasientene hadde vedvarende smerte etter avslutning av stress-testen i både skulder/nakke og hodet, mens migrenepasienter hadde vedvarende smerte i skulder og tinning. Med unntak av en økt EMG-respons i tinning hos migrenepasienter sammenlignet med kontroller, ble det ikke funnet forskjeller i muskelaktivitet i løpet av testen hos noen av gruppene, og

muskelaktivitet var ikke relatert til smerteutvikling. Tensjonshodepine-pasienter hadde vedvarende økt muskelaktivitet i skulder etter avslutning av stress-testen, i motsetning til kontroller og migrenepasienter.

Kardiovaskulære responser på stress var ikke forskjellig hos migrenepasienter og kontroller. Hos tensjonshodepine-pasienter ble det observert en manglende hjerterate-adaptasjon under stresstesten, og det ble funnet en tendens til en forsinket systolisk blodtrykksrespons. Det ble også observert en vedvarende vasokonstriksjon i fingrene etter stress i tensjonshodepine-pasientene, og stress-indusert smerte var relatert til mindre vasokonstriksjon etter stress-testen.

Tensjonshodepine-pasientene hadde en mindre nivå-forandring i kortisol i løpet av stress-testen sammenlignet med kontroller og migrenepasienter, noe som ikke er rapportert tidligere. Hos migrenepasientene ble det funnet lavere nivå av noradrenalin, og noradrenalin-nivåene var inverst korrelert med smerteresponser i disse pasientene.

Konklusjoner

Våre resultater viser at bade migrene- og tensjonshodepine-pasienter responderte forskjellig på stress-testen i forhold til kontrollene. De forhøyede og vedvarende smerteresponsene og potensieringen av smerte under testen som ble observert i tensjonshodepinegruppen, er i samsvar med tidligere hypoteser om sentral sensitisering i disse pasientene, og vedvarende muskelaktivitet antyder at sensitiseringen involverer både smerte- og muskelaktiverende nervebaner. Våre data på migrenepasientene tyder på en mer begrenset regional smertesensitisering og viser at nakkesmerter kan tenkes å være en utløsende faktor til eller et forvarsel-symptom på stress-utløste migreneanfall hos noen av pasientene. Muskelaktivitet målbar fra hudoverflaten antas å ikke være medvirkende i utvikling av smerte hos migrene- og tensjonshodepine-pasienter.

Data på smerte, kardiovaskulære variabler og kortisolnivå tyder på at tensjonshodepine-pasienter har avvikende stress-adapterende mekanismer sammenlignet med kontroller og migrenepasienter. Disse mekanismene involverer sannsynligvis regulering av det

kardiovaskulære systemet, den hypotalamiske-hypofyse-adrenokortikale akse og smerte-regulerende systemer.

Hos migrenepasienter tyder et lavere noradrenalin-nivå og en negativ korrelasjon mellom noradrenalin og smerteresponser på at sympatonevrale responser på stress er knyttet opp mot smerteutvikling hos disse pasientene.

Abbreviations and definitions

ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
BF	Skin blood flow
BP	Blood pressure
CRF	Corticotropin-releasing factor
CTTH	Chronic tension-type headache
DBP	Diastolic blood pressure
EMG	Electromyography
EPQ-N	Eysenck personality questionnaire
ETTH	Episodic tension-type headache
FB	Feedback-instructed rest
HPA	Hypothalamo-pituitary-adrenocortical
HPLC	High performance liquid chromatography
HR	Heart rate
ICHD-1	International classification of headache disorders, first version
ICHD-2	International classification of headache disorders, second version
PAG	Periaqueductal grey matter
PPP	Platelet-poor plasma
PRP	Platelet-rich plasma
RMS	Root mean square
SBP	Systolic blood pressure
SIA	Stress-induced analgesia
TTH	Tension-type headache
UIR	Uninstructed rest
VAS	Visual analogue scale

General introduction

Stress is in several studies mentioned as the most frequent trigger of headache. Nevertheless, the exact mechanisms by which stress induces headache is poorly understood and relatively few studies with rigorous scientific methods have been performed. In the present study an experimental model was used to study muscular, cardiovascular and biochemical responses to standardized low-grade stress and investigate relationships between these responses and pain in the head and shoulder/neck areas.

Headache

Very few people will go through life without experiencing headache in some form. The causes of headaches are many and diverse. Headache may occur as an isolated phenomenon, as part of an acute symptom complex (e.g. migraine) or as a symptom of an underlying disorder (e.g. brain tumours, stroke or metabolic dysfunction). The International Headache Society presented the first classification system for headaches in 1988 (International classification of headache disorders, ICHD-1). It was revised in 2004 (ICHD-2), and this system has become the standard for headache diagnosis [1, 2]. The system divides headaches into two main categories: primary and secondary headaches. Primary headaches are disorders where the headache is not caused by another disorder, whereas the pain is caused by an underlying disorder in secondary headaches. In this thesis the two most common primary headache disorders are investigated: migraine and tension-type headache (TTH).

Migraine

Migraine is an episodic headache condition associated with several non-headache symptoms affecting more than 10% of all adults [3]. Among the most commonly reported trigger factors for migraine (Figure 1) are stress, menstruation and hunger [4-6]. The migraine attack is divided into four phases: the prodrome phase, aura phase, headache phase and headache resolution phase. The prodrome phase occurs in about

60% of migraine patients, often several hours or even days before headache onset. The prodrome can consist of psychological (mood or behavioural changes) or neurological (photophobia, phonophobia) symptoms, as well as other symptoms such as stiff neck, thirst and food cravings [7]. The aura phase occurs in 20-50% of patients (depending on aura frequency) and consists of a complex of neurological symptoms (visual, sensory or aphasic) preceding the start of the headache. According to the ICHD-2 [2], the headache phase occurs within 60 minutes after the end of the aura phase. The headache phase is characterised by a unilateral, throbbing pain of a moderate to intense severity, which is aggravated by moderate physical activity. Headache onset is usually gradual but may be relatively acute in some cases, and lasts for 4-72 hours in adults. The headache is also accompanied by other features, such as nausea, vomiting, photophobia and phonophobia. In addition to the symptoms used in diagnostic evaluation, many patients suffer from other systemic symptoms possibly indicating autonomic dysfunction (such as blurry vision, anorexia, hunger, tenesmus, diarrhoea, abdominal cramps, polyuria and pallor of the face). Most patients experience increased scalp tenderness or stiffness, and tenderness in the neck. In addition, localized oedema of the scalp, face or under the eyes also occurs in some patients. Impairment of concentration, depression, fatigue, anxiety, nervousness and irritability are common. During the resolution phase, the pain gradually decreases. The patients may feel tired, irritable and listless, and some suffer from impaired concentration and mood changes. The ICHD-2 diagnostic requirements for migraine are presented in Appendix A, Table A1.
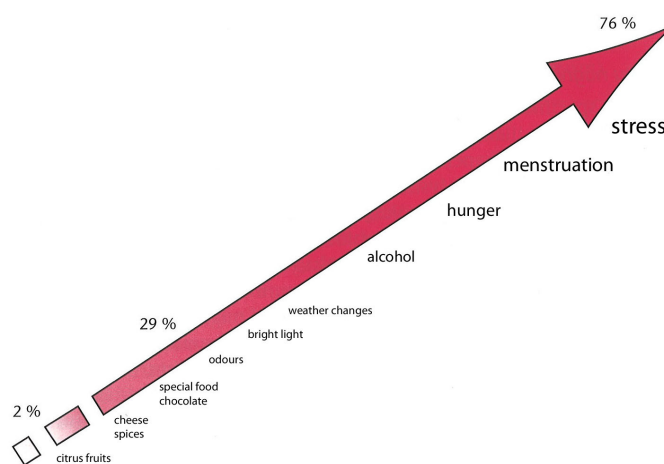

Figure 1. The most common triggers for migraine (figure adapted from Dahlöf et al. [8]).

Tension-type headache

TTH is the most common type of primary headache, with a one-year prevalence in the general population around 42% [3]. Similarly to migraineurs, TTH patients often report stress and hunger as triggering factors for headache, in addition to noise [4, 5]. The headache is characterized by a dull, non-pulsatile feeling of tightness, pressure or constriction, usually of a mild or moderate intensity. The pain is usually bilateral, but varies in location. The headache is, unlike migraine headache, not aggravated by physical activity and not accompanied by nausea, vomiting, phono- or photophobia. TTH was originally thought to be associated with abnormal muscle tension, as defined in the earlier Headache Classification of the Ad Hoc Committee [9]. The involvement of muscle tension in TTH has been the subject of a long-lasting debate, which has still not been resolved. In the ICHD-1, there were two subforms of TTH; with and without associated pericranial muscle disorder. The latter was defined as either increased tenderness on palpation or with pressure algometer, or as increased EMG levels in pericranial muscles. Since such muscle disorders have been difficult to verify, the new classification (ICHD-2) only recognizes subforms with or without pericranial *tenderness*. In the diagnostic criteria TTH is further divided into episodic (ETTH; infrequent (<1 day/month) and frequent (1-14 days/month)) and chronic (CTTH; ≥15 days/month) conditions. The ICHD-2 diagnostic requirements for TTH are presented in Appendix A, Table A2.

The concept of stress

The concept of stress and how it can best be defined in a scientific and medical context have been debated for years. Hans Selye popularized the concept with his theory of stress as a condition shared by all organisms in their interaction with the environment: "Stress is the non-specific response of the body to any demand upon it" [10]. According to this theory, various stimuli would elicit the same stress response, through what he called the General Adaptation Syndrome. This theory has since been discarded by most researchers, as it has been shown that the body reacts in a differentiated manner to various stress stimuli.

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According to the prevailing theory, stress is defined as a condition in which expectations, whether genetically programmed, established by prior learning, or deduced from circumstances, do not match the current or anticipated perceptions of the internal or external environment, and this discrepancy between what is observed or sensed and what is expected or programmed elicits patterned, compensatory responses [11]. In simpler terms, stress causes a shift in the homeostasis of the body, and the body will respond to this in an attempt to re-establish homeostasis. The homeostasis is monitored by homeostats with feed-back inhibited regulatory systems, which will turn off the response when homeostasis is established. Based on information from these homeostats, a wide range of responses can be initiated, regulated not only in the area of effect but also in magnitude. Repeated disruption of such homeostatic systems may result in permanent changes to the absolute value of the monitored variable, possibly causing negative effects on the body.

An abnormal and prolonged stress response has been hypothesized to cause chronic pain and other subjective health complaints. Karasek and Theorell presented a model suggesting that psychological demands at work can predict health [12], and Melin & Lundberg hypothesized that certain work conditions may lead to prolonged endocrine and muscular responses after work in some individuals [13]. McEwen introduced the concept of "allostatic load" [14, 15], which is the strain on the body produced by repeated ups and downs of physiologic systems responding to a stressor. If this allostatic load continues to accumulate, for instance during long-term stress, allostatic response systems (such as the sympathetic nervous system and the hypothalamo-pituitary-adrenocortical (HPA) axis) may become overstrained and dysfunctional, leading to overexposure of stress hormones having adverse effects on the body and possibly leading to disease. A similar model has been presented by Ursin and Eriksen [16, 17]. Their "Cognitive Activation Theory of Stress" (CATS) focuses more on the psychological or cognitive aspects of a stressor, where sustained arousal (a similar concept of McEwen's allostatic load) possibly due to a lack of coping with the stressor, may cause "normal" subjective complaints related to the musculoskeletal system and the gastrointestinal tract, as well as fatigue, dizziness, headaches etc., to become disabling illnesses in some people.

Autonomic activation to stress

Stress can initiate a variety of different autonomic responses in the human body. These responses are regulated through a variety of different systems. Among the systems most commonly linked to stress are the sympathetic and parasympathetic nervous systems, and the HPA axis [11, 18]. The degree of activation of the different systems depends on the nature and intensity of the stressor. Physical and psychological stress will usually increase sympathetic activity and inhibit parasympathetic activity in the relevant subsystems. Should the stressor become severe or uncontrollable, adrenaline will be released into the blood stream from the adrenal medulla through sympathomedullar innervation. In addition, adrenocorticotropic hormone (ACTH) and cortisol will be released through the HPA axis. The sympathetic nervous system regulates blood flow distribution either through secretion of noradrenaline as a neurotransmitter in sympathoneural synapses or through noradrenaline circulating in the blood stream [19]. Most of the circulating noradrenaline is released from spillover of post-ganglionic sympathetic nerve endings, though around 20% is released from the adrenal medulla. In addition to regulating skin and muscle blood flow, sympathetic neurones also innervate glandular structures and parenchymal organs such as liver, kidneys, bladder and reproductive organs. The HPA axis, through its release of cortisol, regulates energy metabolism, suppresses reproductive, immune and digestive functions, enhances vascular reactivity, inhibits inflammation and promotes analgesia, among other things [20, 21].

Autonomic activity is controlled through different centres in the brain. Among the most important ones are the paraventricular nucleus in the hypothalamus, which receives input from limbic and frontal structures and regulate activity of both the sympathoneural and neuroendocrine systems [22, 23]. Central autonomic regulation can cause sympathetic activity to differ in different organs, for instance sympathetic discharge to skin and muscle are different during exercise, thereby causing different effects on blood flow in these organs [24, 25]. The sympathetic nervous system and the HPA axis, in addition to sharing control centres, also interact with each other [11]. For instance, adrenaline levels are regulated by HPA activity, since induction of

phenylethanolamine-*N*-transferase, an enzyme involved in the synthesis of adrenaline, is dependent on cortisol levels near the adrenal medulla. Another example is the fact that noradrenaline will stimulate release of corticotropin-releasing factor (CRF) from the paraventricular nucleus of the hypothalamus. CRF will in turn activate other autonomic and adrenocortical responses. This rather complex regulation of both systems allows a wide range of responses to various stressors.

In addition to activating the autonomic nervous system, stress also seems to increase somatomotor activity [26, 27], though less is known about the specific mechanisms. Central autonomic nerves can affect the somatomotor system indirectly through emotionally motivated motor behaviour [28], but some evidence suggests that central autonomic nerves may also affect motoneurones directly through an "emotional motor system" [29, 30].

Sympathetic activation and pain

The role of peripheral sympathetic activity in pain modulation is still subject to debate. It is generally agreed that in healthy tissues sympathetic activity has little or no effect on pain [31-33], though one study has reported thermal hyperalgesia in healthy skin tissue [34]. However, in conditions with neural injury or neural sensitization, the sympathetic nervous system can modulate pain [32, 35-38], and a condition of sympathetically maintained pain (SMP), where pain is dependent on sympathetic neural activity, has been found in a condition known as complex regional pain syndrome (CRPS) [39, 40]. In relation to migraine and TTH, some symptoms indicate autonomic dysfunction, but it is still unclear whether pain is related to sympathetic activity in such patients [41]. It may seem unlikely that either TTH or migraine headache reflect a neuropathic type of pain, but C-fibers can be activated by endogenous sympathetic bursts or injection of norepinephrine [39]. In other (unilateral) headache syndromes (e.g. the trigeminal autonomic cephalgias) there seems to be a clear association between headache attacks and autonomic hyperactivity [42, 43]. Trigeminal dysfunction is also implicated in migraine and cutaneous mechanical and thermal allodynia has been demonstrated during migraine attacks [44]. Pain sensitivity is increased in TTH suggesting abnormal

pain processing [45-47]. It accordingly makes sense to study autonomic activation in relation to pain responses in migraine and TTH.

Central components of the sympathetic nervous system may also contribute to pain inhibition. In acute stressful conditions, the phenomenon of stress-induced analgesia (SIA) may be induced [48, 49]. An important pathway in regulating the effects of SIA is the circuit from the periaqueductal grey matter (PAG) through the rostral ventromedial medulla (RVM) to the dorsal horn [50]. Contrary to the effects of acute (novel) stress, one study suggests that chronic stress may cause increased pain, but this study did not use a standardized model to induce stress, so the validity is questionable [51]. Increased nociceptive responses to both physical and emotional stress have also been reported in several animal studies [52].

Stress in headache patients

Stress is a known trigger of headache attacks in both migraine and TTH patients [4, 5, 53-55]. One of the physiological responses to cognitive stress is increased muscle activity [26, 27], but the pathophysiological role of muscle activity in headache has not been fully established. Muscle activity has been hypothesized to be a part of TTH pathophysiology, and many studies involving TTH patients have investigated the relationship between stress and muscular activity [56]. However, results have been conflicting, with some studies reporting higher electromyographic (EMG) activity at baseline and/or during stress compared to healthy controls [57-63], while others report normal EMG activity [64-69].

Stress also affects the cardiovascular system. Cardiovascular responses to short-lasting acute stress have been measured in headache patients, but inconsistent findings have been reported for both migraine [70-76] and TTH [70, 73, 75, 77-80].

As mentioned previously, stress increases sympathoneural and adrenomedullar activity and it may also activate the HPA axis, but it is not known if this activation is correlated with pain and headache development during a stressful task. Sympathetic activity has

been measured by means of biochemical markers in both migraineurs [81-84] and TTH patients [82, 85]. Elevated plasma cortisol has been reported in e.g. depression [86], a condition often associated with both migraine [87] and TTH, especially CTTH [88-90]. A trend towards elevated cortisol levels has been reported in migraine and chronic tension-type headache in one study [91], but the cortisol response to low-grade cognitive stress has to our knowledge not been studied in headache previously.

Some of these studies investigated biochemical effects of short-lasting stress from stressors such as cold pressor tests, tilt tests and mental arithmetic tests. It may be argued that these short-lasting stressors are of limited relevance with respect to long-lasting, low-grade stressors often reported to induce headaches in daily life. Our stress model attempts to imitate the conditions of working in a stressful office environment, while still upholding the controllable factors of a laboratory setting. By using such a model we aimed to subject the participants to low-grade stress comparable to what they experience during normal working conditions.

Study aims

Stress is a known trigger of headache, but the involved mechanisms are still under debate. Most previous laboratory studies investigating responses to stress in headache patients have used models with short-lasting stress, often of a more intense nature. Also, studies investigating how headache patients recover from stress are scarce. In this thesis a low-grade, long-lasting cognitive stress model was used, and both responses to stress and recovery from stress were studied. The stress-test was performed in a laboratory setting imitating a real-life office environment, offering more external validity than previous standardized tests on both animals and humans (such as the cold pressor test, tilt tests and mental arithmetic tests). The stressor used in the studies of this thesis has previously been shown to induce measurable responses in muscle activity in both healthy controls and in patients with headache or chronic pain syndromes [92-97].

A priori hypotheses were focused on the issue of establishing whether there were differences between migraine, TTH and controls in their response and recovery patterns to cognitive stress regarding pain, muscle activity, autonomic activity and endocrine responses, and whether pain responses (and recovery) correlated with muscular and autonomic response and recovery variables. The study design was primarily descriptive (more focus on effect- and response-pattern estimation than on testing model-based specific hyptheses) and exploratory in nature, aiming to investigate possible links between several types of physiological responses to stress and development of pain in these patients. The general a priori aims are listed below:

1) To investigate the temporal relationship between muscular activation, cardiovascular and biochemical changes and pain development in migraine and TTH patients.
2) To explore potential differences in stress-induced muscular, cardiovascular and biochemical responses and recovery patterns between migraine, TTH patients and controls.

Methods

Subjects

Forty-four healthy control subjects and 40 patients with headache participated in this study. Twenty-two of the headache patients were diagnosed with migraine and 18 were diagnosed with TTH. Twelve of the TTH patients had the chronic form. Background data on subjects included in the study are shown in Table 1. All headache patients were recruited from the Dept. of Neurology, St. Olavs University Hospital, and diagnosed according to the ICHD-1 from 1988 [1]. Among the control subjects no one had had headache or musculoskeletal pain for more than one day per month. Exclusion criteria were: neoplastic disease, hypertension, infectious disease, metabolic, endocrine or neuromuscular diseases, significant psychiatric disorders, connective tissue disorder, tendinitis, recent significant accident or injury, pregnancy, daily medication (e.g. neuroleptics, antiepileptics, Ca^{2+}-blockers, β-blockers, antidepressants), and significant associated diseases affecting either the heart, lungs, cerebrovascular system, or the central or peripheral nervous system. Migraineurs with TTH more than 7 days per month were also excluded. Controls were recruited from workplaces in Trondheim.

Table 1: Background data on subjects included in the study.

Diagnostic group	Controls (n=44)	Migraine (n=22)	TTH (n=18)
Gender ratio (F:M)	35:9	20:2	9:9
Mean age (range)	39.0 (19-61)	41.2 (20-60)	34.7 (19-52)
Mean number of years with headache (range)	-	20.1 (7-37)	8.7 (0-32)
Number of subjects with chronic headache (%)	-	4 (19.0)	12 (75.0)
Mean duration (h) of headache attacks (range)*	-	30 (1-72)	-
Number of subjects with aura (%)	-	12 (57.1)	-
Mean general tension (VAS) (range)	29.7 (0-84)	36.0 (1-87)	26.5 (0-65)
Mean EPQ-N score (SD)	7.0 (4.2)	9.1 (4.0)	7.5 (5.1)
Number of subjects who smoke (%)	12 (35.3)	7 (31.8)	2 (12.5)
Body-mass index (SD)	25.0 (3.5)	23.8 (3.6)	24.7 (4.4)

* One migraine patient had some attacks of short duration.

The project was approved by the Regional Ethics Committee. All participants gave written informed consent and received NOK 500 (USD 75) for transport expenses and inconvenience. The participants were provided with written information concerning the aim of the study prior to the day of the stress test. The aim of studying pain and headache was mentioned, but the information focused on the practical details of the procedure. Therefore, pain expectancy [98] probably contributed little to pain and, if present, would be similar in patients and controls, since the laboratory personnel were blinded as to the diagnostic status.

Questionnaire and interview

Patients arrived at our facility around 8 a.m. and underwent a structured interview concerning headaches and musculoskeletal complaints (distribution, severity, and duration) prior to the stress test. The interview was conducted in a calm and relaxed manner with the subject sitting comfortably, and lasted around 30 min. One of the interview questions was: "Please state the level of general tension you have felt during the last 2-3 months", and the response was scored on a visual analogue scale (VAS) with endpoints: not tense – very tense. The neuroticism index of the Eysenck Personality Questionaire (EPQ-N) scores was also calculated from the questionnaire (Table 1). Two questions in this questionnaire dealt with symptoms of depression, and there were no group differences in the answers to these questions. At the end of the interview, the first blood sample was drawn by venipuncture.

Participants also kept a headache diary for 7 days before and after the stress test. Thirteen of 22 migraineurs reported a migraine attack within two days before the stress test, while 12 patients reported an attack within two days after the stress test.

Electromyographic recordings

Muscle activity was measured by bilaterally recorded surface EMG (electrode diameter 6 mm, fixed distance between the two poles 20 mm). The system noise was $\pm 1.5\ \mu V$

(root mean square, RMS). EMG was filtered (10-1250 Hz), stored on a digitizing tape recorder (Earth Data 128), and A/D converted (ADInstruments Powerlab 16S, sampling rate 2 kHz) for calculation of RMS values (100 ms running time window). Sharp transients and electrocardiographic artifacts were eliminated with a median filter. Bilateral recordings were performed over the frontalis, temporalis, neck (splenius captis), and trapezius muscles. Frontalis electrodes were placed on a vertical line, crossing the pupil with the inferior electrode 10 mm above the upper border of the eyebrow. The inferior temporalis electrode was placed 10 mm lateral to the external angle of the orbit while the second electrode was placed vertically above the first. The neck electrode was placed at the C2 level just at the edge of the trapezius muscle about 35-40 mm laterally while the second electrode was placed directly below. The medial trapezius electrode was placed 10 mm lateral to the midpoint of a line connecting the acromion and the spinous process of C7 while the lateral electrode was placed on the same line. The ground electrode was placed on the C7 spinous process. Figure 2 shows an overview of how the equipment was mounted on the subjects.

Figure 2. A photo showing the recording equipment fastened to the test subject. The photo was made for illustratory purposes only, and the subject did not participate in the actual study (Private photo).

Physiological recordings

Cardiovascular activity was measured by continuous recording of non-invasive finger BP (Portapres, TNO Biomedical Instrumentation, Amsterdam, The Netherlands) [99] and skin BF in the thumbs (Moorlab, time constant 0.02 s, low-pass filter 22 kHz; Moor Instruments Ltd, Devon, England). The BP cuffs were mounted on the intermediate phalanx on the left middle and ring fingers. Finger skin BF was measured bilaterally with the electrodes (fibre separation 0.5 mm) placed on the volar side of the distal phalanx (pulp) of the thumbs. The average value from the left and right thumb was used for analysis, because no significant side difference was found. Signals were sampled at 200 Hz. Heart rate and BP was calculated with the Beatscope 1.0 software (TNO, Amsterdam, The Netherlands).

Respiration was recorded with a thermistor (Embla S-AF-010, Flaga, Reykjavik, Iceland) below the nose with active elements in each nostril and in front of the mouth, but respiration frequency was not analysed in this study due to technical difficulties (7 controls, 8 migraineurs and 2 patients with TTH had corrupted respiration rate data).

Biochemical analyses

Blood was collected into EDTA-vacutainers and immediately placed in ice-water or into vacutainers without an anti-coagulant. Non-coagulated blood was centrifuged for 10 min at 300 g (at a temperature of 4 $^{\circ}$C) to obtain platelet-rich plasma (PRP). After withdrawing an adequate sample of PRP for catecholamine analysis and platelet counting, samples were centrifuged again for 10 min at 3000 g (4 $^{\circ}$C) to obtain platelet-poor plasma (PPP). Serum was collected after 30 min coagulation, by centrifugation at 1500 g, 10 min, at room temperature. All samples were stored at -80 $^{\circ}$C prior to analysis. Plasma catecholamines were extracted by adsorption to aluminium oxide [100] and analysed by high performance liquid chromatography (HPLC) (Merck Hitatchi LaChrom system, Darmstadt, Germany) with electrochemical detection. Catecholamines were separated on a LiChroCART 250-4 column containing LiChrospher 100 RP-18 (5 µm) (Merck, Darmstadt, Germany) using sodium acetate

buffer (pH 4.8) and methanol (8.5 vol%) as eluents [101]. External standards were used for calculation of sample catecholamine concentrations. Cortisol concentrations in serum samples were determined using a competitive enzyme immunoassay kit (R&D Systems, Abingdon, United Kingdom). Serum samples were diluted 8-fold, processed and analysed by absorbance reading (Titertek Multiscan, Titertek, Alabama, USA) at 405 nm, according to the manufacturer's procedure. Catecholamine analyses were done for 27 controls, 19 migraineurs and 14 TTH patients. Cortisol analyses were done for 24 controls, 17 migraineurs and 13 TTH patients. Adrenaline levels in PRP suggested degradation, probably by monoamine oxidase-B [102] and were not included in the statistical analysis.

Stress-test procedure

The subjects were seated in an ordinary office chair without armrests (Figure 2) and performed a two-choice reaction-time test presented on a PC monitor for 60 minutes [93]. The test involved a grid (7 columns by 5 rows, Figure 3) in which a large and a small square were placed randomly [103]. The subject was then presented with a suggestion on how to move the small square to superimpose it on the large square (for instance, "two up, four right"), and the subjects responded by pressing either "right" or "wrong" on a panel before them with their right index or ring fingers, respectively. Then the positions of the squares were changed, and a new suggestion was displayed. The subjects were instructed to carry out the assignment as fast and correctly as possible, and the computer provided feedback on performance by informing whether the answer was correct or not, and how fast the trial was performed (very slow, slow, normal, fast or very fast) [104]. The "normal" response for each subject was determined as the mean response time during a 5-minute trial period.

2 up 4 right
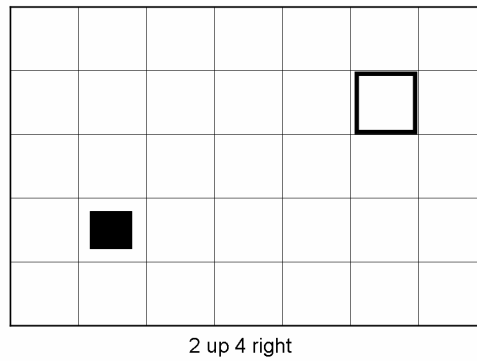
Figure 3. The cognitive task used in the stressor involved a grid (7 columns by 5 rows) in which a large and a small square were placed randomly and the subjects were presented with a suggestion on how to make the small square superimpose over the large square.

Before the stress test was started, the procedure was explained and electrodes were mounted. This lasted for about 45-60 min. A mini trial was also performed to familiarize the subjects with the procedure. Laboratory temperature was kept at 24.5 ± 1 ^{o}C. The recording was started and short maximal voluntary contractions were performed on each pair of muscles twice (frontalis muscle – raising eyebrows, temporalis – clenching teeth, neck – pushing head back against resistance, trapezius – pushing extended arms upwards against resistance at 45° angle out from the body). This was done because we initially intended to present EMG data as a percentage of maximal muscular force [26, 103]. However, the variability between the two maximal contractions in the frontalis area proved to be too large for reliable estimates of maximal force (results not shown), and it was decided to use absolute EMG values for calculating EMG variables instead. After the maximal voluntary contractions, the technician told the subject to relax and then left the room. The recording proceeded with 5 minutes uninstructed rest (UIR) followed by 5 minutes active, instructed rest with visual EMG feedback (FB). During the FB period the technician instructed the subject in a calm, quiet manner on how to relax the muscles more efficiently based on the patient's own EMG data shown on the computer screen. FB was used to assess a "zero" level for EMG. FB-data are shown in figures but were not included in the statistical analysis because it was decided that UIR probably was a more realistic "real-life" baseline. The cognitive task was then performed for one hour (800-1500 trials), followed by 30 minutes recording during rest. The patients were asked to relax while seated and to

move as little as possible during the recovery period. After the UIR and FB periods, at 10-minute intervals during the cognitive task, and at 10-minute intervals during the recovery period, the subjects were asked to mark on a VAS scale their level of pain (no pain – worst bearable pain). The different locations of pain corresponded with the positions of the EMG electrodes [93]. No patient had to be excluded because of headache attacks during the test. Levels of tension, fatigue, and sleepiness were also scored (VAS) by the patient during the test. Venous blood was sampled again immediately after the stressful task. The two blood samples were therefore taken with an interval of 2-3 hours. The stress test was performed in a quiet room with no distractions. Also, patients were able to relax before the first blood sample, and both the nurse and the technician involved in the stress test were instructed to act in a quiet, calm manner at all times. So, while we were unable to control for stressful events that the subjects might have experienced prior to arriving at our facility, we were careful to avoid unnecessary stress after arrival. Figure 4 shows an overview of the test-day procedure.
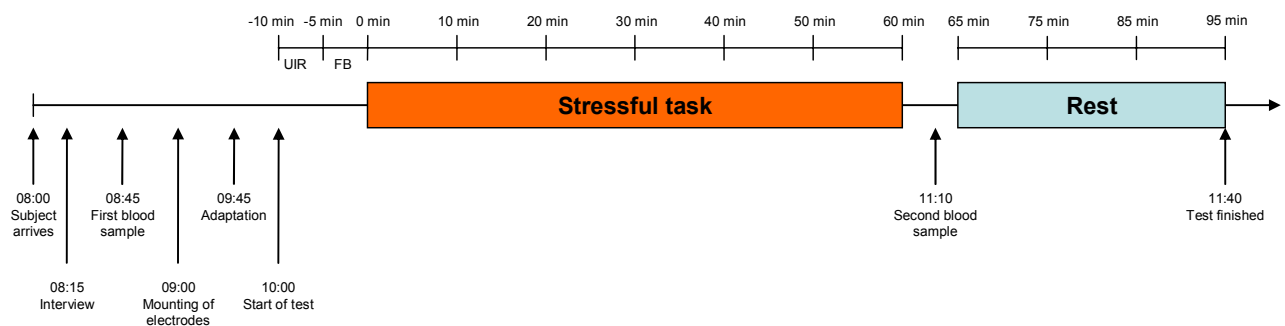
Figure 4. Overview of the test-day procedure.

Data analysis

For UIR, FB and each 10-minute interval during the cognitive test and during recovery we calculated mean EMG RMS values in µV, mean values for systolic blood pressure (SBP), diastolic blood pressure (DBP), HR and finger BF. Levels of plasma

noradrenaline, platelet noradrenaline, plasma adrenaline and serum cortisol, as well as plasma platelet levels, were calculated before and after the stress test. These data were used in the calculations of the different response and recovery variables used in the statistical analyses.

Response variables:

Pain and tension responses: In paper I, pain responses were calculated for each location as the difference between maximal reported pain during the test (irrespective of right or left side) and baseline pain. We discussed whether we should use the mean pain value or the median value during the test in the variable definition instead of the maximal pain, but this was discarded because it was decided that the highest level of pain induced by the stress test was clinically most relevant (a subject in pain will be most conscious of the body part with the greatest pain whether mild pain in other regions is present or not).

To decrease the number of statistical analyses required, only one pain response variable was used for correlation analyses in papers II and III. The variable was defined as the maximal pain response: the largest pain response out of eight location and side-specific responses (the left or right side (maximal value – baseline) for each of the four muscle locations). The tension response in paper II was defined in the same way as to the pain response. In papers II and III the pain and tension responses were only used in correlation analyses.

EMG and cardiovascular responses: In paper I the EMG responses used were defined as the difference between the average EMG value during the test and the UIR value (average of 60 minutes during stress - UIR). In paper II, response variables were calculated for SBP, DBP, HR and finger skin BF. The cardiovascular responses were defined in a similar way as the EMG responses (average of 60 minutes during stress - UIR). Unlike pain, which generally kept a steady rise throughout the test, EMG and cardiovascular development was more diverse, with some variables being stable and some both increasing and decreasing throughout the test. Thus the average during the

stress period was used to calculate the response variable as this was thought to give a more reliable estimate of the physiological state of the subjects during the test.

Biochemical changes: Since we only had two measurements (pre- and post-test), and we lacked a biochemical measurement during the stress period, we could not define a biochemical response variable in the same way as for pain and cardiovascular variables. The term "biochemical change" was therefore used to describe the difference in concentration after the test compared to levels before the test.

Recovery variables:

Pain and tension recovery: To evaluate the patients' recovery from pain in paper I, the number of subjects who did not return to their starting pain was counted for each location at 75 and 95 minutes (early and late recovery respectively). For papers II and III, where pain variables were used in correlation analyses, a different strategy was chosen; pain recovery was defined as the difference between the minimal pain during the recovery period and the baseline value for each location. From the eight recoveries calculated for each patient (left and right side of trapezius, splenius, temporalis and frontalis), the worst recovery value (the largest difference between the recovery period and baseline) was chosen as the point of interest and were defined as the pain recovery because we considered worst recovery as most clinically relevant to study, analogous with the choice for the pain response variable. Tension recovery was defined in the same way as the pain recovery.

EMG and cardiovascular recovery: EMG recovery variables were defined similarly to pain recovery in paper I, i.e. early and late recovery variables were used. However, unlike pain responses, it was considered more relevant to study the temporal evolution of EMG value in the recovery period, because EMG responds fast to stress onset. Therefore, early recovery was defined as the difference between the mean value during the first 10 min of the recovery period (65-75 min) and the UIR baseline value, and late recovery was defined as the difference between the mean during the last 10 min of the recovery period (85-95 min) and UIR baseline.

In papers II and III, cardiovascular recovery variables were only used in correlation analyses, so in order to reduce the number of analyses required, early and late recovery variables were not used. Instead, mean cardiovascular recovery variables (difference between mean level during the whole recovery period and the UIR period) were used in analyses involving SBP, DBP, HR or BF.

Statistical analysis

The strategy for statistical analyses changed throughout the process of writing papers I-III. Therefore, some of the statistical methods used in paper I were not used for similar analyses in papers II and III. Below a summary is given of the statistical strategies and the reasoning behind them. A two-tailed significance level of <0.05 was considered significant in all papers. *P*-values within a range of 0.05-0.10 were considered as trends.

Baseline differences: In paper I, baseline differences in pain and EMG were analysed using the Mann-Whitney U test. In papers II and III, analysis of variance (ANOVA) was used to evaluate group differences in cardiovascular and biochemical baseline values.

Development during and after the stress test: The Mann-Whitney U test was also used to evaluate group differences in pain and EMG responses and recovery in paper I. In addition, repeated measures ANOVA was used to investigate change over time for each muscle location (each group separately), and group differences were evaluated by time × group interactions in three different ANOVA tests for each muscle (controls vs. migraine, controls vs. TTH and migraine vs. TTH). In paper II we wanted to increase the temporal resolution during and after the test in the statistical analysis. Therefore, different repeated measures ANOVA models with selected dependent cardiovascular variables were applied to explore different parts of the stress response and recovery curve: 1) F_2-model y = (baseline, 0-10 min) for the early stress response, 2) F_6 model: y = (0-10 min, 10-20 min, 20-30 min, 30-40 min, 40-50 min, 50-60 min) for the late response (adaptation) during ongoing stress, 3) F_3 model: y = (65-75 min, 75-85 min,

85-95 min) for recovery after stress. A similar model has been used to study patients with fibromyalgia syndrome and shoulder/neck pain [96]. The ANOVA models were corrected for non-sphericity by reduced degrees of freedom with Huyhn-Feldts method. Three-group ANOVA models were used as the primary analysis, followed by three two-group ANOVA models for the differences between controls and migraine, controls and TTH, and migraine and TTH respectively. Group differences in tension response and recovery (summary variables) were explored using the Mann-Whitney U-test. In paper III, an F_2 repeated measures ANOVA model (pre-test and post-test time points) was used to assess biochemical change before and after the stress test.

Correlation analyses: Spearman's rank order correlation analyses were used to explore associations between pain, tension, EMG, cardiovascular and biochemical responses and recovery in papers I-III. Because part of the study was considered mainly to be exploratory we did not apply corrections for multiple comparisons.

Summary of papers

Paper I

Leistad RB, Sand T, Westgaard RH, Nilsen KB, Stovner LJ. 2006. "Stress-induced pain and muscle activity in patients with migraine and tension-type headache." Cephalalgia 26(1): 64-73.

Development of pain and EMG activity in the trapezius, splenius, temporalis and frontalis muscles were analysed. The main findings were higher pain responses in the temporalis and frontalis areas (with similar trends for trapezius and splenius) and more potentiation of pain during the test when comparing TTH patients with controls. Migraine patients developed more pain in the splenius and temporalis areas than controls, and pain responses were more regional (more pain in the splenius and trapezius compared to the temporalis and frontalis) in migraine, while TTH patients had a more generalized pain (no differences between the four areas). TTH patients had delayed pain recovery in all areas compared to controls, while migraine had delayed pain recovery in the trapezius and temporalis areas. The temporalis EMG response was increased in migraineurs compared to controls, but there were no other differences in EMG responses between the diagnostic groups, and EMG responses were not correlated with pain responses. TTH patients had delayed EMG recovery in the trapezius compared to controls and migraineurs. The results suggested that (probably central) sensitization of pain pathways and the motor system are important in TTH, while less pronounced, more regional trigeminocervical sensitization seems to be important in migraine. Surface-detectable muscular activation did not appear to be causal for pain during cognitive stress in migraine or TTH.

Paper II

Leistad RB, Sand T, Nilsen KB, Westgaard RH, Stovner LJ. "Cardiovascular responses to cognitive stress in patients with migraine and tension-type headache." Submitted.

In this paper we analysed the physiological data acquired from the stress test described in paper I. Cardiovascular responses to cognitive stress in migraine did not differ from those in control subjects. In TTH patients, a lack of HR-adaptation during stress was found and a trend towards a delayed SBP response during stress was also observed.

Finger BF recovery was delayed after stress and stress-induced pain was associated with less vasoconstriction in TTH during recovery. It was hypothesized that TTH patients have different stress adaptive mechanisms than controls and migraineurs, involving both cardiovascular activation and the pain control system.

Paper III

Leistad RB, Stovner LJ, White LR, Nilsen KB, Westgaard RH, Sand T. "Noradrenaline and cortisol changes to low-grade cognitive stress differ in migraine and tension-type headache." J Headache Pain 8:71-76.

In this paper, biochemical data from the stress test were investigated. TTH patients had significantly less cortisol change to stress than controls and migraineurs, while migraineurs had lower noradrenaline levels in blood platelets compared to controls. Pain responses correlated negatively with noradrenaline levels, and pain recovery correlated negatively with the cortisol change in migraineurs. Pain recovery correlated negatively with the noradrenaline changes in TTH patients. For migraineurs, the lower noradrenaline levels and the negative correlation found between noradrenaline and pain responses suggests that the sympathoneural response to stress appears to be closely linked with pain. On the other hand, TTH patients had no decrease in cortisol in contrast to controls and migraineurs. HPA axis activation during mental stress coping may therefore be abnormal in TTH.

General discussion

The individual findings in this study have been discussed in detail in papers I-III. In this section, implications of the findings in a broader context will be discussed. Methodological weaknesses and suggestions on how to improve the method will also be discussed to a greater extent than has been the case in papers I-III.

Choice of statistical methods

The use of non-parametric statistics vs. parametric statistics changed somewhat during the course of writing the papers. In paper I, Komolgorov-Smirnov's test indicated that pain data (and to some degree EMG data) did not follow a Gaussian distribution. Instead of applying a transformation function to the data, a more conservative approach was chosen, and all data were analysed with non-parametric statistics (with the exception of repeated measures ANOVA, which has no equivalent non-parametric analysis). Another reason for using non-parametric statistics were that self-reported data (such as pain and tension) were considered to be sensitive to individual scaling differences and ceiling and bottom effects, which might influence the symmetry of the data distribution and cause large intra-group variations and skewness. Non-parametric statistics are less vulnerable to such variations [105]. As the cardiovascular data examined in paper II followed a Gaussian distribution, using repeated measures ANOVA in the statistical analysis was unproblematic. However, for correlation analyses non-parametric Spearmans rank order correlations were used, as these correlations involved both cardiovascular data and pain/tension data. The biochemical data in paper III were LN-transformed in order to obtain a Gaussian distribution, and parametric statistics were used to evaluate the data. Correlation analyses followed the same strategy as in papers I and II, and Spearmans rank order correlations were used.

A priori hypotheses were focused on the issue of establishing whether there were differences between headache groups and controls in their response and recovery patterns to cognitive stress, and whether pain responses (and recovery) correlated with EMG and autonomic response and recovery variables. The design did not allow us to

test for specific model-based hypotheses regarding the possible causal interrelationship between variables. Thus, all three studies included in this thesis were to some degree exploratory, as the results will make it easier to generate models and formulate specific testable hypotheses regarding the possible causal association between e.g. autonomic reactivity and pain. As our general statistical strategy involves a relatively large number of comparisons, it could be argued that there is a need for an adjustment (e.g. Bonferroni) to control for type I errors [106]. We chose not to do this, as this would have created other problems, such as an increase in type II errors [107, 108]. Also, as the studies were considered to be mainly hypothesis-*generating* and not so much hypothesis-*controlling*, we believe that findings worthy of further research might be missed by applying too rigid criteria to the statistical analyses.

In order to investigate associations between pain and cardiovascular responses, as well as their temporal relationships, we also performed stepwise backwards multiple regression that was originally planned to be included in paper II (reported in Appendix B). This strategy allowed us to use a set of pain and cardiovascular variables (early and late response/recovery) to compare different multivariate models in an effort to explore possible causal relationships between pain and autonomic/EMG variables. However, reviewers were sceptical to our approach, deeming the analysis as too explorative, and requested more rigid demands to test statistics (i.e lower p-values) and corrections for multiple comparisons. Though we believe that using such a statistical analysis could provide valuable information about the temporal qualities of our measured variables, we accepted the reviewers' criticism and decided to use repeated measures ANOVA analysis instead in paper II.

Methodological considerations

The subjects' perception of their level of stress was not measured directly in this study. Instead, they were asked to report their level of tension. Holte et al. [109] studied the concept of tension in Norwegian subjects with questionnaires and qualitative interviews and found that subjects described tension in terms of both stress-related autonomic symptoms and musculoskeletal activation. A possible reason for this is that the

Norwegian word for tension ("anspenthet") conveys almost the same meaning as the word stress. Because of this, we believe that perceived tension can be used as an estimate of the subjects' level of stress, though in later studies with a similar design we recommend enquiring directly about the subject's level of perceived stress.

A limited sample size and uneven gender distribution in the three groups caused some concern in our study, especially for the biochemical analyses (paper III). There were some problems with recruiting enough patients and controls, resulting in groups that were smaller than intended. Technical difficulties with some of the blood samples decreased the sample size even more. The low group size and uneven gender distribution made gender-specific statistical analyses less meaningful due to low sample power, and we therefore based our analyses on groups consisting both of men and women. Some of our variables could be influenced by gender distribution, as it is known for instance that women tend to report more pain than men [45, 110], and greater responses to stress have previously been found in male cortisol levels and DBP, but not in HR or SBP [111]. In addition, reproductive steroid levels may influence the stress response [112]. However, our data did not show any gender differences when analysing pain, BP, HR, BF or biochemical responses in all subjects irrespective of diagnosis (Appendix C), thus we do not believe that the uneven gender distribution in our study invalidates our results.

The two blood samples were taken with an interval of 2-3 hours. More frequent sampling by a Veneflon® inserted into the cubital vein was considered but discarded because we anticipated that it would interfere with pain, tension, EMG and cardiovascular measurements. In retrospect, the benefits of a more frequent blood sampling might have outweighed the negative factors. It would for instance be possible to perform a similar statistical analysis in paper III as for the cardiovascular variables in paper II, which would have provided a higher temporal resolution of the biochemical changes in the three subject groups during and after the stress test. It should be mentioned that such a strategy would greatly increase the number of samples to be analysed by HPLC (catecholamines) and immunological assay (cortisol), so substantial

additional resources for the biochemical analyses would be required (extra personnel, funding, etc).

The methodology for sampling cortisol from our subjects may have been sub-optimal. Dickerson and Kemeny [113] report that cortisol assessments should be obtained 21-40 min from stressor onset in order to detect the peaks in cortisol levels, while our results were sampled 60 min after the onset of the stressor. A more frequent sampling of blood as described above, would have removed this potential source of error in detecting the size of the cortisol response. Also, due to the circadian rhythm of cortisol, some aspects of the cortisol response to stress might have been hidden since the samples were taken during the morning hours when cortisol levels were decreasing. However, since the test was performed in the morning for all subjects, we find it highly probable that the reported group differences between TTH patients and controls and migraineurs are real. It should nonetheless be considered that future follow-up studies should be performed in the afternoon when cortisol levels are more stable.

Several subjects reported that their level of pain did not return to baseline during the recovery period. This was also the case for EMG and cardiovascular variables. It would have been of interest to increase the recovery period length, so that we could follow their development further. It might have enabled us to differentiate between the subject group recoveries from stress better than the current model, for instance by evaluating in more detail exactly how much time was needed to return to baseline for the different variables.

In order to characterize how the stress test influenced the recorded responses, it would have been of interest to have the subjects come back for a control session, where all parameters were recorded while the patients did nothing except sit quietly. By doing such a control session, we would be able to estimate the response magnitude caused by the cognitive task, and how much of the response that may have been caused by other unknown factors related to sitting in a rather fixed position. Such a control day was not included in the study protocol. However, the fact that responses in general recovered rather quickly upon task cessation in most controls suggests that it is the task itself, and

not sitting down for an extended period of time, that causes pain and physiological effects.

Appraisal of main findings

The development of pain, EMG and cardiovascular variables (paper I and II) leave little doubt that the stress test had an effect on the subjects. Controls had an increase in muscle activity in the trapezius and frontalis regions, as well as an increase in BP, HR and skin vasoconstriction during the test. More than 50% of the controls also had an increase in pain in the trapezius and splenius regions.

The two patient groups differ in their responses to the stressor in several respects, and both groups also differ from controls. During the stress test, TTH patients had increased pain responses (paper I), a lack of HR adaptation and appeared to have a delayed BP response (paper II) and sustained their cortisol levels (paper III). After the test they had delayed pain recovery, delayed recovery of trapezius EMG (paper I) and delayed recovery of finger BF vasoconstriction (paper II). Correlation analyses suggested that a low noradrenaline response was related to delayed pain recovery, as was the observed lack of HR adaptation. Finger BF recovery was also correlated with the maximal pain response. All in all this creates a rather complex picture of how TTH patients respond to stress.

Migraineurs had increased pain responses in the neck and temporalis areas compared to controls, and they had delayed pain recovery in the trapezius and temporalis areas (paper I). They also seemed to have an increased temporalis EMG response compared to controls, but it was not correlated with the increased temporalis pain response, and it was interpreted as a random finding caused by a rather high temporalis EMG baseline in controls. There were no differences in cardiovascular variables when compared to controls (paper II), but migraineurs did have lower levels of noradrenaline (paper III).

Adaptation to stress

The theories of McEwen [14] and Ursin & Eriksen [16, 17] are of particular interest when looking at how headache patients respond to stress. McEwen hypothesized that allostatic load occurs through lack of adaptation to a stressor, or from inability to shut off a stress response – i.e. lack of recovery. Our findings in TTH patients fit well with this theory, as we found both a lack of HR adaptation during stress and delayed skin BF recovery, EMG recovery and pain recovery. McEwen hypothesizes that allostatic load may cause disease, but whether this may lead to TTH is uncertain. It cannot be analysed in the present cross-sectional study, and longitudinal studies would be needed. Our results do suggest, however, that allostatic load may be partly why patients with TTH develop headache from stress. While McEwen focuses both on the response to stress and the recovery thereafter, Ursin & Eriksen focus mainly on inefficient recovery from stress (sustained arousal) as a cause of health complaints. Repeated inability to recover from stress, for instance caused by the subject's working environment, could create a vicious circle resulting in headache or other health complaints. This is confirmed by Sluiter and co-workers who report a connection between the need for recovery from work-related fatigue (presumably caused by stress and other work-environmental factors) and subjective health complaints [114]. Recovery from stress is relatively understudied compared to responses to stress [115], and most studies investigating recovery from stress in migraine and TTH focus on recovery from acute, short-lasting stressors and provide inconsistent results [63, 71, 72, 116]. The studies included in this thesis are to our knowledge therefore the first to investigate recovery from low-grade long-lasting laboratory stress in migraine and TTH patients. Considering our results in conjunction with the models of McEwen and Ursin & Eriksen, it appears that TTH patients have an inadequate defence mechanism against stress, not able to counteract the "threatening" effect the stressor has on the organism, thus leading to pain and prolonged physiological responses (Figure 5).

```
Cognitive          ⟹          Inadequate stress response          ⟹          Inadequate stress recovery
stressor
                                          ⇕                                                    ⇕

                              - Increased pain response                      - Delayed pain recovery
                              - Lack of HR adaptation                        - Delayed trapezius EMG recovery
                              - Blunted BP response                          - Delayed finger BF recovery
                              - Maintained cortisol levels
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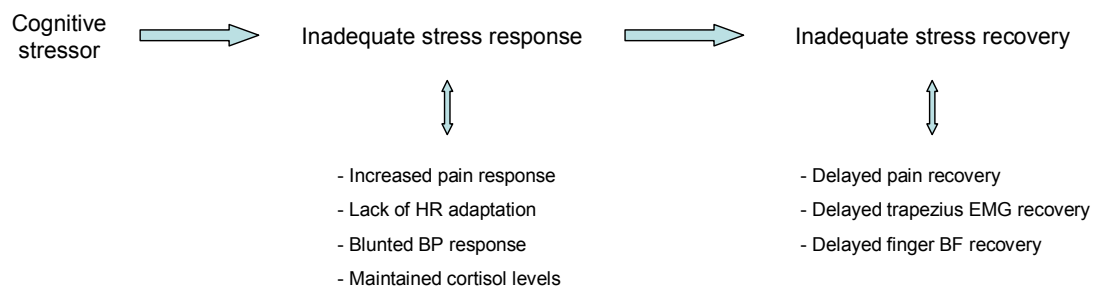
Figure 5. Proposed model for stress-induced responses in TTH based on our findings.

Ursin & Eriksen also suggest that the way a subject copes with a stressor is important for avoiding aggravated subjective health complaints. TTH patients may be more likely to appraise daily situations as stressful, with a tendency towards passive coping, compared to non-headache controls [56]. Because cognitive processing involving the prefrontal cortex can change activity in different parts of the PAG, a difference in stress adaptive mechanisms may influence both the autonomic nervous system and pain control system in several ways, for instance by delaying sympathetic cardiovascular activation [117]. PAG is also important in pain control and central sensitization, possibly explaining allodynia and hyperalgesia to pressure stimuli [47, 118] and the increased stress-induced pain (paper I) in TTH.

There are studies indicating that the way subjects perceive and cope with a stressor influences the HPA activity. A meta-analysis by Dickerson & Kemeny [113] showed that performance tasks perceived as social-evaluative, especially those with uncontrollable factors, provoked a greater release of cortisol than tasks where such factors were not present. Our stress-model involved such social-evaluative aspects, as the subjects received feedback on their performance during the test and they knew they were being monitored through a video camera. There were also uncontrollable factors, since the patients were instructed to sit still and work as fast and correctly as possible, with no way to take breaks or move to relieve themselves from pains or stress. Hence, the fact that TTH patients in our study maintained their cortisol levels during the

stressful task may suggest that they cope differently with the stressor than controls or migraineurs.

Stress-induced muscular activation

The experimental model used in this thesis induced muscular activation in both healthy subjects and patients, as expected from previous experience with this model [92-95]. Our results support previous observations in that increased cephalic muscle activity is not a central part of TTH pathogenesis [64-69]. However, we did find prolonged muscle activity in the trapezius muscle of TTH patients after stress. It is possible that the emotional motor system proposed by Holstege [29, 30], which can cause involuntary modulation of the motoneurone excitability as a response to stressful stimuli, may be responsible for lowering recruitment thresholds of motoneurones in the trapezius during stress [119, 120]. The observed lack of muscle activity recovery could hypothetically be a result of continued low motoneurone recruitment thresholds as a result of a prolonged stress response. It remains to be clarified whether such lack of muscular recovery is a risk factor for future disability or headache chronicity.

Stress-induced pain

In our study pain increased more during the test in TTH patients compared to controls, while the stress-stimulus intensity was assumed to be equal for controls and patients. The lack of differences in EMG responses in TTH patients compared to controls, and the fact that we found no correlation between pain and EMG responses, indicate that muscular activity is not causal to pain in these patients. However, one cannot rule out that pain may be caused by overexertion of low-threshold single motor units in the muscles [27]. Hägg hypothesized that low-threshold motor units may be active even when the overall muscle activity is low [121], based on Hennemans recruitment principle stating that there is a fixed recruitment order of motor units [122]. It would therefore be possible for motor units in the muscle to be active while being undetectable with surface EMG. Pain induced from such motor unit activity would therefore not necessarily be correlated with EMG activity.

The observed "pain potentiation" in TTH patients may reflect a temporal summation of pain. Temporal summation, which is thought to be associated with central sensitization, has previously been shown in muscles in healthy subjects [123]. Central pain sensitization, commonly defined as increased responsiveness of nociceptive neurones, which either outlasts the initiating input or requires a low-level peripheral drive to maintain it [124], has been suggested to be important in both TTH (especially CTTH) [45-47, 125-129] and migraine [130-132]. However, there is some controversy as to whether temporal summation is confirmative of central sensitization, but at least it seems clear that temporal summation is only one of many mechanisms involved in central sensitization [133-135]. Few studies have specifically investigated temporal summation in headache patients, and results are not yet conclusive. Ashina and co-workers recently reported a statistically non-significant tendency for patients with CTTH to achieve a higher degree of temporal summation to suprathreshold pain stimuli compared to controls in the trapezius, temporalis and anterior tibialis muscles [45]. This is in contrast to a study by Fusco et al. who found no difference in temporal summation for CTTH patients [136]. It should be mentioned that the latter study used a painful stimulus on the volar surface of the forearm, a region not associated with pain in TTH patients, and a negative finding here would not rule out a predisposition for temporal summation (and possibly central sensitization) in areas associated with pain in TTH, such as the head and shoulder/neck areas. Temporal summation can only be hypothesized and not confirmed in this thesis. As we do not know the mechanisms behind the stress-induced pain observed in our study, we would require a controlled external painful stimulus to confirm this hypothesis.

In humans, aftersensations (defined as pain perceived after a stimulus has ended) have been found in experimental conditions which induce central sensitization in 2nd or higher order neurones [137]. Though both the time-frame and the nature of the stimulus are very different in our study, our observation of prolonged pain in TTH patients (and migraineurs to some extent) after the stress-test has ended may be interpreted as aftersensations. Increased temporal summation and aftersensations of pain have also been reported in fibromyalgia [138], a group of patients that share many common

clinical, therapeutic and pathophysiologic features with TTH patients [139]. But since aftersensations to painful stimuli were only measured for a few minutes after the stimulus had ended in the studies by Gottrup et al. [137] and Staud et al. [138], it is uncertain whether this phenomenon can be attributed to the >30 min delay in pain recovery we observed in some subjects. Another question that needs to be considered is whether subjects experience the recovery period as "stress-free", i.e. if our stimulus was completely shut off when the test ended. While we interpret the temporal development of pain, tension and cardiovascular variables as indications that the healthy controls did not feel stress after the test ended (as they generally recovered well), it is still difficult to claim that the element of stress was completely removed. In animal models, wind-up (an equivalent of temporal summation observed in humans) induced by high-frequency stimulation of C-fibers have been reported to be maintained by stimulation at low frequencies that normally would not produce wind-up [134]. If some patients did experience low-frequency stimulation even after the test had ended, this could be an alternative explanation of the observed delayed pain recovery in TTH and migraine patients.

Another possible explanation for the increased pain in migraine and TTH patients could be inhibition of endogenous pain-modulatory mechanisms such as SIA. The level of stress required to induce SIA has not been established, and therefore we do not know whether our low-grade stress test would be enough to induce it in our subjects. Previous studies on humans have used stressors such as painful tactile, thermal or electrical stimulation, and the intensity of the stimulation seems to be related to the degree of pain reduction induced. However, some studies (mainly studies involving acupuncture on animals) suggest that uncontrollability and other cognitive aspects of a stressor can also be important in SIA [48]. Nonetheless, assuming that our stressor would be able to induce SIA, one would expect to find an inverse correlation between a sympathetically mediated stress response and increased pain if such a phenomenon were present. In our studies we found an inverse correlation between pre-/post-test levels of noradrenaline and the pain response in migraineurs, suggesting that high sympathetic activity (reflected by noradrenaline levels) may protect against the pain in these patients, as one could expect from SIA. However, low noradrenaline levels in migraineurs suggest low

sympathoneural activity, indicating that these patients, like TTH patients, may also have an inadequate physiological response to stress which is unable to protect against pain. In TTH patients, no correlation between the HR response (assumed to be an indication of a "blunted" stress response in our study) and the pain response was found, nor was there a correlation between noradrenaline changes and the pain response. On the other hand, less vasoconstriction in the recovery period correlated with a high pain response during the test in TTH. A negative correlation between post-test sympathetically mediated BF decrease and test-pain was accordingly observed. Based on our results it is difficult to explain this correlation. One possible explanation could be that pain is causal to inhibited sympathetic vasoconstriction in the skin [140]. Another alternative is that stress-induced sympathetic activity influences central or peripheral pain modulation in TTH patients. It might have been possible to verify if SIA was present or not by administering naloxone, which is known to reverse the effects of this phenomenon. This should lead to increased pain in subjects with a functioning SIA, possibly with less effect in TTH patients if they already have impaired SIA.

A striking feature of the stress response in migraineurs is their increase in neck pain. Neck pain has previously been reported in 75% of migraine patients associated with either the prodrome, headache or postictal phase [141]. Since 50% of our migraine patients reported an attack within one day following the stress test, one may speculate that the stress-induced neck pain acted as a trigger or a prodrome for the migraine attack. The origin of the neck pain is uncertain, but as there was slightly reduced muscular activity, the pain at least does not appear to be related to increased muscular activity.

There were relatively few findings that distinguished migraineurs from controls in our study. Except for an increased temporalis EMG response, there were no differences in muscular activity or cardiovascular variables. This may suggest that migraineurs, during the interictal phase, are less susceptible to the negative effects of stress on general pain and motor systems than TTH patients, though we did observe increased temporalis pain, i.e headache (but not a migraine attack), in these patients. Stress may also still lead to

the initiation of a migraine attack in some patients by more specific effects on the proposed migraine generator in the brainstem [142].

Autonomic dysfunction in migraine has been suggested by several other researchers [82, 143-147]. Based on a literature review and their own studies, Thomsen, Olesen et al. concluded in 1995 that definite sympathetic dysfunction remained to be shown while mild parasympathetic hypofunction with denervation supersensitivity might be present in migraine [143]. We found low noradrenaline levels, suggesting decreased sympathetic activation during cognitive stress, while cardiovascular function was mainly normal. An autonomic imbalance in migraineurs may accordingly be of a small magnitude (see also discussion about HR in migraine in appendix B) and in addition affect subsystems which were not directly investigated in the present study (e.g. involvement of the cranial sphenopalatine parasympathetic nervous system [148] or sympathetic and parasympathetic innervation of the eye [149]). Data from the literature are not consistent, but suggest that more severe stressors differentiate better between migraineurs and controls. The intensity of the stressor of the present model has previously been varied by increasing the demands for reaction time in the stress test [104], but no differences were found in muscle activity and HR as the intensity of the stressor was increased. Offering rewards to increase the motivation for the task did change the responses though [104], so this may be an alternative route to increase stressor responses.

It seems clear that further study on pain mechanisms in headache is required, particularly in conjunction with central sensitization, though sensitization of peripheral structures should also be investigated [150]. Exposing patients to controlled painful stimuli similar to the study by Ashina and co-workers [45], just after the patients has performed a stressful task as in this thesis, could provide interesting insight about the role of stress adaptation and coping in the development of pain and central sensitization. It would also be interesting to vary the intensity and length of the stressor in such a model, in order to investigate how this would influence pain perception (as well as autonomic responses) in these patients. By increasing stressor severity it might also be possible to study if specific physiological changes predict an imminent headache attack.

Prospective long term studies are also needed to test if stress response magnitude or lack of recovery can predict future headache severity or frequency. While adding a pain stimulus after the stressor has ended would compromise some of the external validity of our real life-imitating stress-test (as people seldom receive painful stimuli while working in an office environment), the potential insight to pain and stress mechanisms might nevertheless make such a strategy worthwhile.

Conclusions

The aim of this thesis was to explore how patients with migraine or TTH respond to low-grade cognitive stress in a laboratory setting imitating a real-life office environment. Based on the analysis of how stress influences muscular activation, activation of the autonomic nervous system, and pain, and by studying the extent of physiological recovery after stress, we hoped to acquire new information about the pathophysiology of these headaches.

Both migraineurs and TTH patients responded differently to stress compared to controls. TTH patients reported more pain, and we found a potentiation of pain during the stress test and prolonged pain recovery after the test. Compared to migraineurs, TTH patients had a more generalized increase in pain (both in the neck/shoulders and the head) while migraineurs had higher pain increase in the neck/shoulders compared to the head. Our results support the view that central sensitization of pain pathways is present in TTH patients. Our migraine data also suggest that neck pain may be a trigger or prodrome to migraine attacks in these patients. Muscular activity did not appear to be associated with pain in any of the subject groups.

Cardiovascular data revealed no differences in cardiovascular responses between the three groups. However, a temporally blunted HR response, interpreted as a lack of HR adaptation, was found in TTH patients, and TTH patients also differed in finger BF recovery. Finger BF recovery was also correlated to the pain response. Based on these results we hypothesize that TTH patients have different stress adaptive mechanisms compared to controls and migraineurs involving cardiovascular activation.

As to biochemical effects, TTH patients appeared to have a derangement in the HPA axis. Maintained cortisol levels during stress, which have not been reported before, support the hypothesis of abnormal stress adaptive mechanisms in TTH patients. The cortisol response to cognitive stress has not been studied in migraine before either, and was found to be normal. For migraineurs the noradrenaline levels were lower than for controls, and inversely correlated to the pain response, which may indicate that while

sympathoneural activity (reflected by noradrenaline levels) may inhibit pain in these patients (for instance through endogenous pain-modulatory mechanisms such as SIA), their level of stress-induced activation was not high enough to achieve this. This could, in part, explain their increased pain responses to stress.

The most commonly reported trigger for headache, namely stress, has been relatively little studied by experimental methods with good external validity prior to this study. Previous studies on stress in headache patients have involved stressors such as the cold pressor test, tilt test and mental arithmetic test, all of which are short-lasting and have low relevance to the stressors that headache patients experience in daily life. Based on our results, further studies on how TTH and migraine patients respond to stress and recover from stress, both at a cognitive level (coping) and a physiological level (pain processing and stress-adaptation) are warranted. Despite some shortcomings in our model and the procedure used in this thesis, the work nevertheless represents one of the first attempts to study experimentally the frequently-reported connection between work-related stress and development of pain in headache patients.

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Paper I

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Paper II

Cardiovascular responses to cognitive stress in patients with migraine and tension-type headache

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Abstract

Background: The purpose of this study was to investigate the temporal relationship between autonomic changes and pain activation in migraine and tension-type headache induced by stress in a model relevant for everyday office-work.

Methods: We measured pain, blood pressure (BP), heart rate (HR) and skin blood flow (BF) during and after controlled low-grade cognitive stress in 22 migraineurs during headache-free periods, 18 patients with tension-type headache (TTH) and 44 healthy controls. The stress lasted for one hour and was followed by 30 minutes of relaxation.

Results: Cardiovascular responses to cognitive stress in migraine did not differ from those in control subjects. In TTH patients, a lack of HR-adaptation during stress was found. A trend towards a delayed systolic BP response during stress was also observed in TTH. Finger BF recovery was delayed after stress and stress-induced pain was associated with less vasoconstriction in TTH during recovery.

Conclusions: It is hypothesized that TTH patients have different stress adaptive mechanisms than controls and migraineurs, involving delayed cardiovascular adaptation and reduced pain control system inhibition.

Key words: Migraine, tension-type headache, stress, cardiovascular responses, pain.

Background

Prolonged physiologic (e.g. autonomic) responses to a stressor, or insufficient recovery from stress, may cause disease, chronic pain or other subjective complaints [1-3]. Stress may also trigger headache in both migraine and tension-type headache (TTH) patients [4-7]. In other headache syndromes (e.g. "trigeminal autonomic cephalalgias") there seems to be a clear association between headache attacks and autonomic hyperactivity [8, 9], and migraine symptoms in the prodromal phase and during attacks (e.g. nausea and vomiting) suggest autonomic imbalance also in these patients. Trigeminal and brainstem dysfunction is also implicated in migraine during attacks [10-12], and pain sensitivity is increased in TTH suggesting abnormal pain processing [13, 14]. Because of the known interactions between autonomic and pain control centres in the brainstem (e.g. for the baroreceptor reflex [15]), and because autonomic hyperactivity may sensitize peripheral nociceptors [16], it makes sense to study if autonomic activation to stress is abnormal in migraine and TTH compared to healthy subjects, and if autonomic activation is related to the pain responses in these patients [17].

Cardiovascular responses to short-lasting acute stress have been measured in migraine but no clear pattern emerges [18-24], and data about responses and adaptation to more long-lasting cognitive stress are lacking. For TTH, most studies concerning physiological responses to stressors have focused on muscular activity [25], and studies investigating cardiovascular responses to stressors report inconsistent findings [18, 21, 23, 26-29]. Low-grade long-lasting cognitive stress may be more relevant to daily (e.g. work-related) stress than short-lasting stressors used in previously published studies, such as deep breathing tests, orthostatic tests, the cold pressor test

and mental arithmetic tests. In addition, data about physiologic recovery after stress, which may be of particular importance as disease promoting factors [2, 30], are insufficient in the headache research literature. Since migraine and TTH patients in some cases can have rather similar symptoms [31-33], although they are considered as clearly different syndromes, it was reasonable to include both entities in one study.

We have recently found that migraine and TTH patients have more stress-induced muscle pain and slower muscle pain recovery after long-lasting cognitive stress than controls [34, 35]. This experimental task induces muscular activity and pain in the shoulders, neck and head of patients with migraine [34], TTH [34, 36], cervicogenic headache [37], fibromyalgia [38, 39] as well as in healthy controls [40]. However, muscular activation did not correlate with pain responses and no muscular response differences were found between migraine, TTH and controls [34]. Measuring cardiovascular and skin blood flow (BF) responses to stress and recovery after stress in parallel with pain in migraine and TTH may give insight into other potentially pain-inducing or contributing mechanisms in primary headache disorders.

The main questions were: do the early and the late autonomic activation pattern during stress and the recovery pattern differ in migraine, TTH and headache-free controls? Do the autonomic activation and recovery pattern correlate with increased pain during stress and recovery in migraine and TTH patients? We accordingly recorded blood pressure (BP), heart rate (HR) and skin BF development as well as head and shoulder/neck pain in these subjects during low-grade cognitive stress for one hour followed by 30 minutes of relaxation.

Methods

Subjects

Forty-four healthy control subjects, 35 women (mean age 39.7 years) and 9 men (36.6 years) and 40 patients with headache participated in this study. Twenty-two patients had migraine, 20 women (39.8 years) and 2 men (45.0 years), and 13 of these patients had aura. Eighteen patients had TTH, 9 women (33.8 years) and 9 men (35.7 years). Twelve of the TTH patients had chronic TTH. Detailed subject and headache history data are shown in Table 1. Patients were diagnosed after interview and physical examination by a neurologist according to the International Headache Society classification of headache from 1988 [41]. Control subjects did not suffer from headache or musculoskeletal pain for more than one day per month. Exclusion criteria were: neoplastic disease, hypertension, infectious disease, metabolic, endocrine or neuromuscular diseases, significant psychiatric disorders, connective tissue disorder, tendinitis, recent significant accident or injury, pregnancy, daily medication with neuroleptics, antiepileptics, Ca^{2+}-blockers, β-blockers, antidepressants, and significant associated diseases affecting either the heart, lungs, cerebrovascular system, central or peripheral nervous system. Migraineurs with TTH more than 7 days per month were also excluded. The project was approved by the Regional Ethics Committee. All participants gave written informed consent and received NOK 500 (USD 75) for transport expenses and inconvenience. The participants were provided with written information concerning the aim of the study prior to the day of the stress test. The aim of studying pain and headache was mentioned, but the information focused on the practical details of the procedure.

Questionnaire and interview

A structured interview concerning headaches and musculoskeletal complaints (distribution, severity, and duration) was performed. One of these questions was: "Please state the level of general tension you have felt during the last 2-3 months", and the response was scored on a visual analogue scale (VAS) with endpoints: not tense – very tense. Participants also kept a headache diary for 7 days before and after the stress test. All subjects answered a questionnaire on marital status, weight, stimulant use, exercise habits, and sleep problems (data not shown). With the exception that migraineurs had lower alcohol consumption than controls (Chi-Square test, p=0.034), there were no statistically significant differences in these parameters.

Thirteen of the 22 migraineurs reported a migraine attack within two days before the stress test, while twelve patients reported an attack within two days after the stress test.

Physiological recordings

Muscular activity was recorded with surface electromyography (EMG) bilaterally in the trapezius, splenius, temporalis and frontalis muscles, as described in a previous paper [34]. Autonomic activity was measured indirectly by continuous recording of non-invasive finger BP (Portapres, TNO Biomedical Instrumentation, Amsterdam, The Netherlands) [42] and skin BF in the thumbs (Moorlab, time constant 0.02 s, low-pass filter 22 kHz; Moor Instruments Ltd, Devon, England). The BP cuffs were mounted on the intermediate phalanx on the left middle and ring fingers. Finger skin BF was measured bilaterally with the electrodes (fiber separation 0.5 mm) placed on the volar side of the distal phalanx (pulp) of the thumbs. The average from the left and right thumb was used for analysis, because a significant side difference was not found.

Signals were sampled at 200 Hz. HR and BP was calculated with the Beatscope 1.0 software (TNO, Amsterdam, The Netherlands). Respiration was recorded with a thermistor (Embla S-AF-010, Flaga, Reykjavik, Iceland) below the nose with active elements in each nostril and in front of the mouth, but respiration frequency was not analysed in this study due to technical difficulties (Seven controls, eight migraineurs and two patients with TTH had corrupted respiration rate data).

Procedure

The subjects were seated in an ordinary office chair without armrests and performed a two-choice reaction-time test presented on a PC monitor for 60 minutes [40]. The test involved a grid (7 columns by 5 rows) in which a large and a small square were placed randomly [43]. The subject was then presented with a suggestion on how to move the small square to superimpose it on the large square (for instance, "two up, four right"), and the subjects responded by pressing either "right" or "wrong" on a panel before them with their right index or ring fingers, respectively. Then the positions of the squares were changed, and a new suggestion was displayed. The subjects were instructed to carry out the assignment as fast and correctly as possible, and the computer provided feedback on performance by informing whether the answer was correct or not, and how fast the trial was performed (very slow, slow, normal, fast or very fast) [44]. The "normal" response for each subject was determined as the mean response time during a 5-minute trial period. The subjects were acclimated to the lab environment for 30 minutes, during which the procedure was explained and the recording equipment were mounted. The recording started with 5 minutes uninstructed rest (UIR) followed by 5 minutes active, instructed rest with visual EMG feedback (FB). FB-data are shown in figures but were not included in the

statistical analysis because it was decided that UIR probably is a more realistic "real-life" baseline. The cognitive task was then performed for one hour (800- 1500 trials), followed by 30 minutes recording during rest (recovery period). The subjects were asked to relax while seated and to move as little as possible during the recovery period. After the UIR and FB periods, at 10-minute intervals during the cognitive task, and at 10- minute intervals during the recovery period, the subjects were asked to mark on a VAS scale their level of pain (no pain – worst bearable pain), tension, fatigue and sleepiness. The different locations of pain corresponded with the positions of the EMG electrodes. Figure 1 shows an overview of the test day procedure. No patient had to be excluded because of headache attacks during the test. Venous blood was sampled before the test (immediately after the interview was concluded) and immediately after the stress period (after 60 minutes). Blood sample data will not be reported in this paper.

Some subjects had partly missing data due to technical difficulties: Two controls and two migraineurs had corrupted BP and HR data during the test and recovery period. One control was missing pain data at t_{95min}, while one patient with TTH had corrupted BP, HR, BF, pain and tension data during the recovery period.

Data analysis

Mean values for systolic blood pressure (SBP), diastolic blood pressure (DBP), HR and finger BF were calculated for the UIR and FB period, and for each 10-minute interval throughout the stress test and recovery period. These data were used in statistical ANOVA models (see below).

In order to minimize the number of correlations we also defined summery variables for autonomic response and recovery, and for pain response and pain recovery. Two summary variables were used for each autonomic variable (SBP, DBP, HR and finger BF) in correlation analyses: mean response (average of 60 minutes during stress – UIR) and mean recovery (average of 30 minutes recovery – UIR). The *pain response* was defined as the highest pain response (max pain at $t_{10-60min}$ – pain at t_{0min}) among the eight location- and side-specific responses (trapezius, splenius, temporalis and frontalis muscles, left and right side). The muscle-specific pain data have been published in a previous paper [34]. The minimal pain during recovery was used first to calculate eight location- and side-specific pain recoveries (minimal pain at $t_{75-95min}$ – pain at t_{0min}). Thereafter, the highest among these eight location- and side-specific pain recoveries was defined as *pain recovery*. These definitions were chosen because the highest (worst) pain during test (and the least complete recovery) was considered to most clinically relevant. Tension response and recovery were defined identically to the pain variables. Pain and tension variables are shown in Table 1.

Statistical analysis

Baseline values were compared with univariate ANOVA (F_1 models). Repeated measures ANOVA time × group interaction was used to explore differences in response patterns between groups. We do not report group-factor statistics in the present exploratory study because baseline values did not differ between groups (see results). Three different models with selected dependent variables were applied to explore different parts of the stress response and recovery curve. To examine how the novelty of the stressor influenced the subjects, the first 10 min and the baseline was

compared in a F_2-model (y = (baseline, 0-10 min)). This was described as the early (acute) stress response. After the first 10 min it was assumed that the novelty aspect of the stressor were gone, and we used a model named F_6 with six repeated dependent variables (y = (0-10 min, 10-20 min, 20-30 min, 30-40 min, 40-50 min, 50-60 min)) to examine how the subjects adapted to the stressor. This was described as the late stress response. A F_3-model with three dependent variables (y = (65-75 min, 75-85 min, 85-95 min)) was used to examine how fast and complete the subjects recovered from the stressor. The ANOVA models were corrected for non-sphericity by reduced degrees of freedom with Huyhn-Feldts method. Three-group ANOVA models were used as the primary analysis, followed by three two-group ANOVA models for the differences between controls and migraine, controls and TTH, and migraine and TTH respectively. Intra-group contrasts were explored by post-hoc Student's paired t-test. Group differences in pain and tension response and recovery (summary variables) were explored using Mann-Whitneys U-test. Univariate Spearman's rank order correlation analyses were used to explore associations between pain, tension and cardiovascular responses and recovery (summary variables). As our general statistical strategy involves a large number of comparisons, some might argue that there is a need for a multiple-comparison adjustment to control for type I errors [45]. We chose not to do this, as this would create other problems, such as an increase in type II errors [46, 47]. Also, as the studies were considered to be mainly hypothesis-*generating* and not so much hypothesis-*controlling*, we believe that findings worthy of further research might be missed by applying too rigid criteria to the statistical analyses. A two-tailed significance level of <0.05 was considered significant in the statistical analyses. *P*-values within a range of 0.05-0.10 were defined as trends.

Results

There were no statistically significant differences between the three subject groups when comparing physiological baseline values (see F_1 values in Table 2). Inspecting Figures 2 and 3, it appears that SBP, DBP and HR increased more abruptly and then decreased (i.e a "spiked" shape in Figures 2 and 3) at the start of the stressor in controls and migraineurs, but not in patients with TTH.

Cardiovascular responses to cognitive stress

ANOVA F_2 analyses did not reveal any significant time × group interactions between the groups with regard to the initial (early) BP, HR or BF stress responses.

The late HR response pattern during ongoing stress from 0-10 to 50-60 min was significantly different between the three groups (see F_6 time × group interaction value in Table 2) since HR adaptation in TTH differed significantly from HR adaptation in controls (Table 3). HR levels were stable in TTH patients whereas HR decreased after the initial response in controls (Figure 3).

The SBP response tended to increase from the early (0-10 min) to the latest (50-60 min) part of stress (Student´s paired t-test, p=0.051) in TTH, while responses were stable in migraine and in controls (p>0.66; Figure 2). SBP tended to decrease from 0-10 to 10-20 min in migraine patients (Student's paired t-test, p=0.050) while no difference was found in TTH (p=0.97). Significant ANOVA time × group differences were not found in SBP and DBP adaptation during the stress test however (F_6 models in Table 2 and 3),

Cardiovascular recovery after cognitive stress

TTH patients had a significant F_3 time $\times$ group interaction for finger blood flow during the recovery period, compared to controls and migraine patients (Table 3). Figure 3 shows that finger blood flow in TTH patients continued to decrease throughout the recovery period, whereas this did not happen in the other groups.

Relationship between pain, tension and cardiovascular responses and recovery
In patients with TTH, mean finger BF recovery were related to the maximal pain response ($r_s = 0.49$, p=0.047), meaning that a high pain response was related to less finger BF reduction in recovery. There were no correlations between maximal pain responses and BP or HR responses, or between pain recovery and mean cardiovascular recovery, in any of the diagnostic groups. Pain responses were abnormally large while pain recovery were delayed in TTH patients compared to controls while perceived tension responses did not significantly differ between groups (Table 1, Figure 4). TTH patients also had significantly less recovery from tension compared to controls. There were no correlations between tension and cardiovascular responses and recovery for any of the three groups.

Discussion

Controls, and to a certain degree also migraineurs, responded to the stressor in the present study with a rapid increase followed by a relatively fast decrease in BP and HR, giving the curve a spike-like shape. However, in TTH patients, the SBP, DBP and HR profiles increased slowly and did not decrease during the stress test. A trend towards a different SBP profile was found when comparing the first and last 10-min interval in controls and TTH. The significant lack of HR-adaptation during stress reflects the lack of a HR-spike (followed by a decrease in HR) in TTH. A reduced early cardiovascular response to mental stress, with the heart rate response inversely correlated to the pain response, was found for fibromyalgia patients in a study with a similar design [48]. Cardiac (HR) adaptation to mental stress has previously been reported in healthy students [49], while deficient cardiac adaptation to calculative mental stress has been found in migraine patients [50]. The migraine patients in our study did not show signs of deficient HR adaptation to stress. One may interpret the lack of an acute spike at the start of the cognitive task and the lack of HR adaptation as evidence of a deficient adaptive mechanism (or decreased autonomic excitability) to low-grade cognitive stress in TTH patients.

HR in migraineurs recovered as much during the relaxation phase as controls. This is in accordance with another study [19] which did not show a difference in HR recovery between students with migraine and controls after three minutes of mental arithmetic, although the authors reported faster recovery in peripheral resistance in migraine compared to controls. On the other hand, Holm *et al.* [20] found that migraineurs had delayed HR recovery after four minutes of stressful speech-

preparation. Methodological differences make it difficult to compare short-lasting cognitive stress with the one-hour test we applied.

The observed skin blood flow reduction during test is probably related to a gradually increasing sympathetic vasoconstrictor tone to skin arterioles and AV-shunts during cognitive stress [51]. However, we did not find any differences in finger BF development during the test between the three groups. This is in accordance with previous studies that have utilized finger temperature and pulse amplitude as indirect measures of finger blood flow during short-duration stress with generally negative results in TTH [25] and migraine [19].

We did find a delayed finger BF recovery profile *after* stress in TTH compared to controls and migraineurs. Another study has previously reported prolonged skin vasoconstriction in TTH (earlobe pulse volume and finger temperature) [29], which is in accordance with our findings. In addition, TTH patients had delayed pain recovery (Table 1) and delayed EMG recovery in the trapezius area [34]. Our findings in general fit well with the theoretical models of Eriksen & Ursin [1] and McEwen [2]. Our lack of HR adaptation in TTH is in accordance with McEwens concept of "allostatic load" which causes lack of adaptation to stress. Furthermore, the lack of skin BF recovery in TTH fits well both with the concept of "sustained arousal" in the model of Eriksen & Ursin, and with the concept of a prolonged response to a stressor in McEwens model.

The role of the autonomic nervous subsystems in TTH is not clear [25]. Because muscular blood flow in tender points is decreased in TTH [52], and because we observed increased skin vasoconstriction (reduced BF) during recovery after stress, which was correlated to low pain response during stress, it is possible that sympathetic dysregulation is involved, for instance as hyperactivity or hypersensitivity in the central autonomic network which again may be linked to increased central pain inhibition. It is also possible to explain this effect through pain-induced inhibition of sympathetic vasoconstriction in the skin however [53].

Recently, decreased muscle blood flow during muscle exercise was found in fibromyalgia patients, suggesting that muscle ischemia contributes to pain in these patients [54]. However, we were not able to measure intramuscular blood flow in the present study. Muscle blood flow is regulated differently from skin blood flow [55] and the direct relevance of observed skin blood flow changes to the relationship between muscle blood flow and pain perceived as muscular is accordingly uncertain.

Also in migraine, there are still many uncertainties about the role of autonomic nervous subsystems [17, 19, 24, 56, 57]. Some studies report autonomic dysfunction in migraineurs, such as orthostatic hypotension, noradrenergic or adrenergic hypofunction etc. [58-63], but not all studies report such autonomic dysfunction [64-66]. Many past studies have used procedures such as deep breathing tests, orthostatic tests, cold pressor tests and isometric work tests (sustained handgrip) and these responses are not directly comparable with autonomic response to cognitive stress of long duration used in the present study.

Cephalic and intracranial vessels may be regulated differently from peripheral vessels. Painful stimuli to tooth pulp induce a blood flow increase in orofacial areas [67]. In chronic TTH patients, previously published data indicate cranial vasodilatation [68]. In migraine, cephalic pulse amplitude may increase during a mental task in migraine [18] but results are not consistent across studies [19], and both deficient and normal vasoactivity has generally been reported in migraine [66]. Our results support the view that dysfunctional peripheral blood flow regulation is not a substantial part of migraine pathophysiology.

Although we did not measure perceived stress in this study, we believe that the measured perceived tension is an indirect measure of the level of stress. The Norwegian word "anspenthet" describe a feeling of general psychological and muscular tension perceived in stressful situations [69]. Tension responses did not differ, thus the level of stress seemed to be comparable between groups. However, TTH patients had a significantly less recovery from tension, indicating an inability to unwind after the stressor is removed [70].

As to what is perceived as stressful, TTH-patients may be more likely to appraise daily situations as stressful, with a tendency towards passive coping, compared to non-headache controls [25]. Because cognitive processing involving the prefrontal cortex can change the activity in the different parts of the periaqueductal grey matter (PAG), a difference in stress adaptive mechanisms may influence both the autonomic nervous system and pain control system in several ways, for instance by delaying sympathetic cardiovascular activation [71]. PAG is also important in pain control and

in central sensitization, possibly explaining allodynia and hyperalgesia to pressure stimuli [72] and the increased stress-induced pain in TTH (Table 1, Figure 4).

In conclusion, we report a lack of HR adaptation to stress in TTH patients, as well as a delayed finger skin BF recovery after stress and a correlation between finger skin BF recovery and the pain response. Also, TTH had an increase in SBP from the first 10 min to the last 10 min of the stress test, whereas controls and migraineurs did not. Autonomic responses to cognitive stress were not abnormal in migraine. We hypothesize that TTH patients have different stress adaptive mechanisms compared to controls and migraine patients, involving both cardiovascular activation and the pain control system. The motor system is also involved in responses to stress [73-75], and low-threshold motor unit activity may contribute to local metabolic changes and muscle pain [76, 77]. However, because no associations between muscle activity and pain activation was found in a previous study [34] , the present results suggest that cardiovascular responses are more closely linked to pain control than reflexes regulating muscle activity in TTH patients.

List of abbreviations:

BF   Blood flow

BP   Blood pressure

DBP   Diastolic blood pressure

EMG Electromyography

FB   Feedback period

HR   Heart rate

PAG   Periaqueductal grey matter

SBP   Systolic blood pressure

TTH   Tension-type headache

UIR   Uninstructed rest period

VAS   Visual analogue scale

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions:

RBL participated in acquiring data from the stress test, performed the statistical analyses and drafted the manuscript. TS participated in the design of the study, assisted in the statistical analyses and helped draft the manuscript. KBN participated in the design of the study, acquired data from the stress test and helped draft the manuscript. RHW participated in the design of the study. LJS participated in the design of the study and helped draft the manuscript.

All authors read and approved the final manuscript.

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Tables & Figures:

Table 1: Background data on subjects included in the study. Pain/tension responses and recoveries are given in group means.

Diagnostic group	Controls (n=44)	Migraine (n=22)	Tension-type headache (n=18)
Gender ratio (F:M)	35:9	20:2	9:9
Mean age (range)	39.0 (19-61)	41.2 (20-60)	34.7 (19-52)
Mean number of years with headache (range)	-	20.1 (7-37)	8.7 (0-32)
Number of subjects with chronic headache (%)	-	4 (19.0)	12 (75.0)
Mean duration (h) of headache attacks (range)*	-	30 (1-72)	-
Number of subjects with aura (%)	-	12 (57.1)	-
VAS pain response (range)	15.4 (0-66)	22.7 (0-54) [2,3]	38.5 (3-88) [1]
VAS pain recovery (range)	3.4 (0-47)	4.4 (0-19) [2,3]	16.4 (0-74) [1]
VAS tension response (range)	21.2 (-13-82)	27.6 (-1-70)	32.7 (0-76) [2]
VAS tension recovery (range)	13.0 (-11-75)	18.5 (-14-67)	26.4 (-16-65) [1]

* One migraine patient had some attacks of short duration.
[1] Patients vs. controls, $p \leq 0.05$. [2] Patients vs. controls, $0.05 < p < 0.1$. [3] Migraine vs. TTH $0.05 < p < 0.1$ (Mann-Whitney tests).

Table 2: Physiological mean values (SD) measured at baseline, during mental stress (0-60 min) and during the recovery period (65 -95 min).

		Controls Mean (SD)	Migraine Mean (SD)	Tension-type headache Mean (SD)
Systolic blood pressure (mmHg)	Baseline	112.7 (15.4)	114.0 (14.4)	106.1 (16.5)
	0-10 min	125.4 (17.0)	128.7 (20.0)	117.1 (18.4)
F_1 (2, 79) = 1.49, p=0.23	10-20 min	122.7 (16.7)	125.7 (18.7)	117.1 (20.1)
F_6 (7.0, 269.0) = 1.19, p=0.31	20-30 min	122.5 (15.3)	125.7 (17.0)	117.9 (17.8)
F_2 (2, 77) = 0.42, p=0.66	30-40 min	122.6 (16.0)	128.3 (17.8)	120.0 (17.6)
F_3 (3.3, 128.7) = 0.60, p=0.63	40-50 min	123.1 (14.6)	126.0 (16.3)	121.2 (17.7)
	50-60 min	125.1 (14.9)	127.6 (14.7)	122.8 (17.0)
	65-75 min	124.5 (16.5)	122.0 (13.6)	114.5 (19.7)
	75-85 min	121.9 (14.0)	120.6 (10.4)	115.7 (16.8)
	85-95 min	123.8 (14.5)	123.1 (11.1)	116.8 (17.8)
Diastolic blood pressure (mmHg)	Baseline	62.3 (11.5)	62.4 (8.6)	60.8 (11.3)
	0-10 min	71.8 (13.4)	71.4 (10.8)	67.4 (10.2)
F_1 (2, 79) = 0.13, p=0.88	10-20 min	70.1 (14.0)	70.1 (9.3)	67.5 (12.1)
F_6 (7.0, 268.9) = 0.77, p=0.62	20-30 min	69.7 (11.0)	69.8 (8.6)	67.7 (9.4)
F_2 (2, 77) = 0.77, p=0.47	30-40 min	70.9 (11.6)	71.9 (9.9)	69.3 (10.4)
F_3 (2.8, 107.9) = 0.34, p=0.78	40-50 min	70.4 (10.6)	71.3 (9.4)	70.1 (10.7)
	50-60 min	71.4 (10.2)	71.3 (8.1)	70.3 (9.8)
	65-75 min	72.4 (13.7)	71.5 (10.1)	67.6 (12.3)
	75-85 min	69.7 (9.2)	69.4 (7.3)	67.3 (8.2)
	85-95 min	71.4 (9.5)	70.4 (6.7)	67.6 (8.5)
Heart rate (beats/min)	Baseline	71.1 (8.6)	73.9 (13.3)	73.8 (9.1)
	0-10 min	74.6 (10.3)	78.0 (12.4)	75.4 (9.5)
F_1 (2, 79) = 0.76, p=0.47	10-20 min	73.6 (9.2)	77.6 (12.7)	75.8 (9.4)
F_6 (4.0, 156.9) = 2.87, p=0.025	20-30 min	73.2 (8.8)	76.9 (12.6)	76.0 (9.6)
F_2 (2, 77) = 1.3, p=0.28	30-40 min	72.3 (8.5)	76.8 (12.2)	76.0 (9.6)
F_3 (3.9, 148.2) = 1.03, p=0.39	40-50 min	72.1 (8.6)	77.1 (12.5)	76.0 (9.3)
	50-60 min	72.1 (8.3)	76.9 (12.6)	75.9 (9.4)
	65-75 min	69.0 (7.5)	73.7 (11.6)	71.8 (9.0)
	75-85 min	69.1 (7.9)	72.8 (11.5)	71.1 (9.2)
	85-95 min	68.8 (7.7)	73.2 (11.4)	71.2 (8.6)
Finger skin blood flow (arbitrary units)	Baseline	278.5 (112.0)	306.2 (114.7)	283.3 (72.2)
	0-10 min	247.5 (121.9)	271.1 (110.4)	246.5 (70.5)
F_1 (2, 81) = 0.52, p=0.60	10-20 min	250.6 (130.3)	273.2 (103.6)	246.0 (74.3)
F_6 (5.0, 189.8) = 049, p=0.78	20-30 min	246.0 (126.8)	273.4 (108.0)	248.8 (66.2)
F_2 (2, 81) = 0.07, p=0.94	30-40 min	249.2 (125.8)	262.9 (107.4)	239.5 (72.1)
F_3 (3.9, 157.0) = 2.3, p=0.07	40-50 min	237.1 (127.4)	265.4 (113.1)	228.6 (78.8)
	50-60 min	228.7 (120.4)	250.4 (102.3)	208.8 (85.3)
	65-75 min	214.9 (104.6)	258.1 (114.8)	207.6 (96.0)
	75-85 min	228.6 (110.6)	287.3 (119.1)	189.6 (105.4)
	85-95 min	211.0 (105.6)	262.1 (101.3)	182.9 (90.1)

F_1: Oneway ANOVA F-statistic comparing baseline values between groups. F-statistic for group $\times$ time interaction in repeated measures ANOVA models is also tabulated for three different models in the left column: F_2: Model for the *early response to stress,* with two intervals during the early stage of the stress test (baseline and 0-10 min). F_6: Model for *adaptation or potentiation during ongoing long-lasting stress,* with six intervals during the stressful task (from 0-10 to 50-60 min). F_3: Model to detect *fast versus slow recovery patterns* with three intervals during recovery (65-75, 75-85 and 85-95 min). p: Probabilities (degrees of freedom in parentheses) was adjusted for non-sphericity with Huyhn-Feldt's method. Significant interactions and trends in bold.

Table 3: F-statistic for group × time interaction in two-group repeated measures ANOVA models.

	Controls vs Migraine	C vs TTH	M vs TTH
Systolic blood pressure	F_2 (1, 60) = 0.33, p=0.57	F_2 (1, 58) = 0.22, p=0.64	F_2 (1, 36) = 0.97, p=0.33
	F_6 (3.3, 198.9) = 0.44, p=0.74	F_6 (3.5, 200.2) = 1.54, p=0.20	F_6 (3.5, 125.8) = 1.99, p=0.11
	F_3 (1.68, 102.3) = 0.24, p=0.75	F_3 (1.6, 89.7) = 1.06, p=0.34	F_3 (1.9, 67.4) = 0.47, p=0.62
Diastolic blood pressure	F_2 (1, 60) = 0.04, p=0.85	F_2 (1, 58) = 1.70, p=0.20	F_2 (1, 36) = 0.76, p=0.39
	F_6 (3.2, 190.9) = 0.22, p=0.89	F_6 (3.5, 202.7) = 1.27, p=0.29	F_6 (3.4, 121.7) = 0.82, p=0.50
	F_3 (1.4, 84.5) = 0.06, p=0.88	F_3 (1.3, 76.8) = 0.59, p=0.50	F_3 (1.6, 57.0) = 0.47, p=0.59
Heart rate	F_2 (1, 60) = 0.06, p=0.80	F_2 (1, 58) = 1.98, p=0.17	F_2 (1, 36) = 2.85, p=0.10
	F_6 (2.0, 122.2) = 1.46, p=0.24	**F_6 (2.0, 115.8) = 5.06, p=0.008**	F_6 (2.1, 75.0) = 1.48, p=0.23
	F_3 (1.9, 117.0) = 1.83, p=0.17	F_3 (2.0, 113.4) = 0.83, p=0.44	F_3 (1.9, 65.1) = 0.12, p=0.88
Finger skin blood flow	F_2 (1, 64) = 0.06, p=0.81	F_2 (1, 60) = 0.15, p=0.70	F_2 (1, 38) = 0.01, p=0.94
	F_6 (2.5, 161.1) = 0.31, p=0.79	F_6 (2.2, 133.1) = 066, p=0.53	F_6 (2.7, 100.6) = 0.57, p=0.61
	F_3 (1.9, 124.3) = 0.66, p=0.52	**F_3 (2.0, 119.5) = 3.21, p=0.04**	**F_3 (1.9, 72.7) = 3.47, p=0.04**

F_2: Repeated measures ANOVA model with two intervals during the early stage of the stress test (baseline and 0-10 min). F_6: Model with six intervals during the stressful task (0-60 min). F_3: Model with three intervals during recovery (65-95 min). p: Probabilities (degrees of freedom in parentheses) was adjusted for non-sphericity with Huyhn-Feldt's method. Significant interactions in bold.
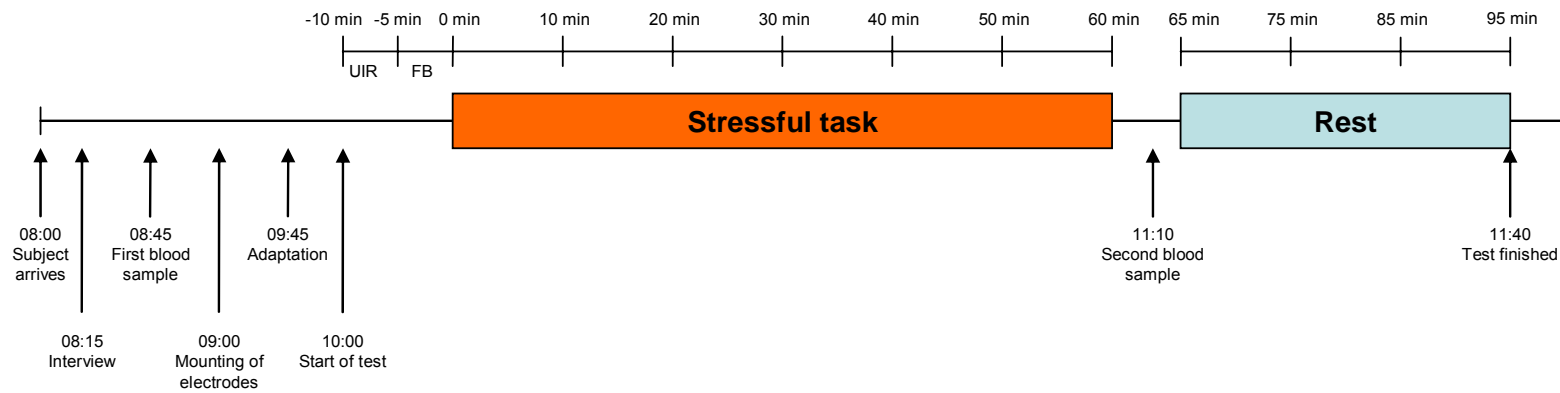
Figure 1: Overview of the test-day procedure. The subjects arrived at 08:00 and started with a structured interview, followed by the first blood sample. At approximately 09:00 the electrodes were mounted, and after a short adaptation period, the stress test started at 10:00. The stress test (incl. UIR and FB rest periods, stress period and recovery period) lasted for approximately 1h 40min.
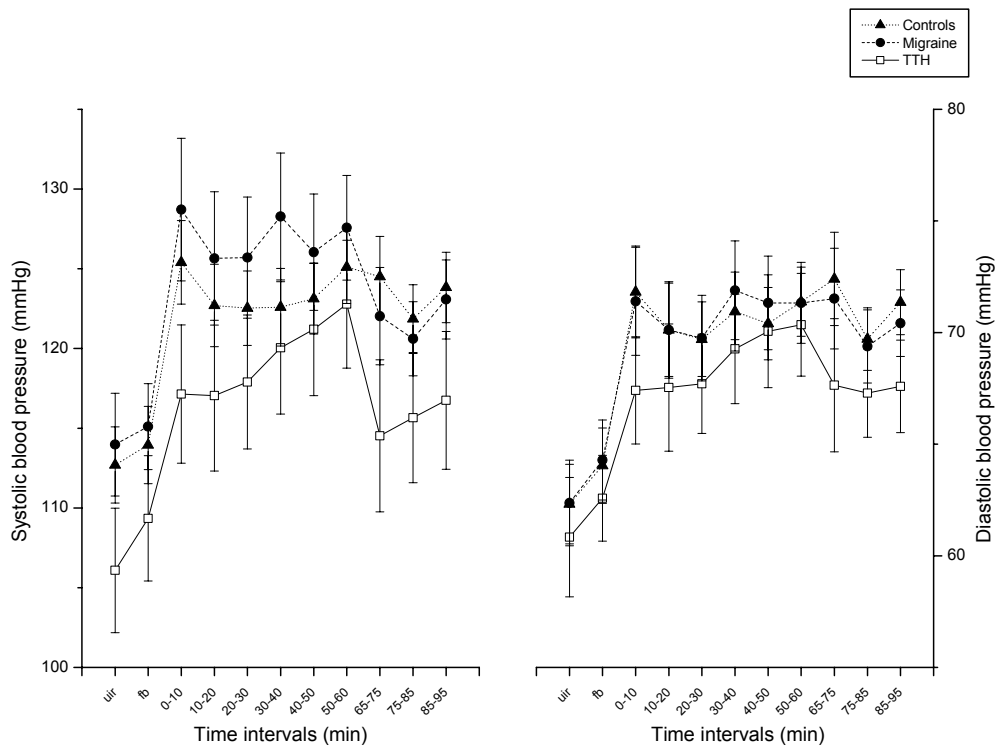
Figure 2: Systolic and diastolic BP development throughout the stress test and recovery period. Values are given as group means (SEM). UIR: Uninstructed rest period (baseline EMG). FB: EMG feedback aided rest period. 0 – 60: During the cognitive stress test. 65 – 95: Relaxation period after the test.
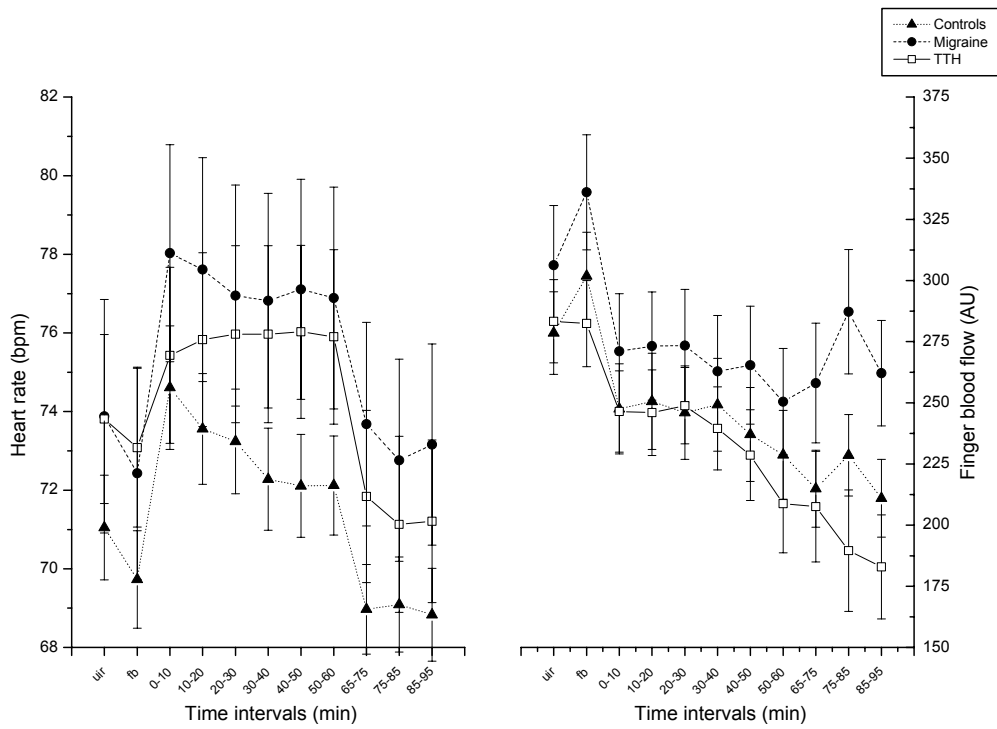
Figure 3: Heart rate and finger blood flow development throughout the stress test and recovery period. Values are given as group means (SEM). UIR: Uninstructed rest period (baseline EMG). FB: EMG feedback aided rest period. T=0 – 60: During the cognitive stress test. T= 65 – 95: Relaxation period after the test.
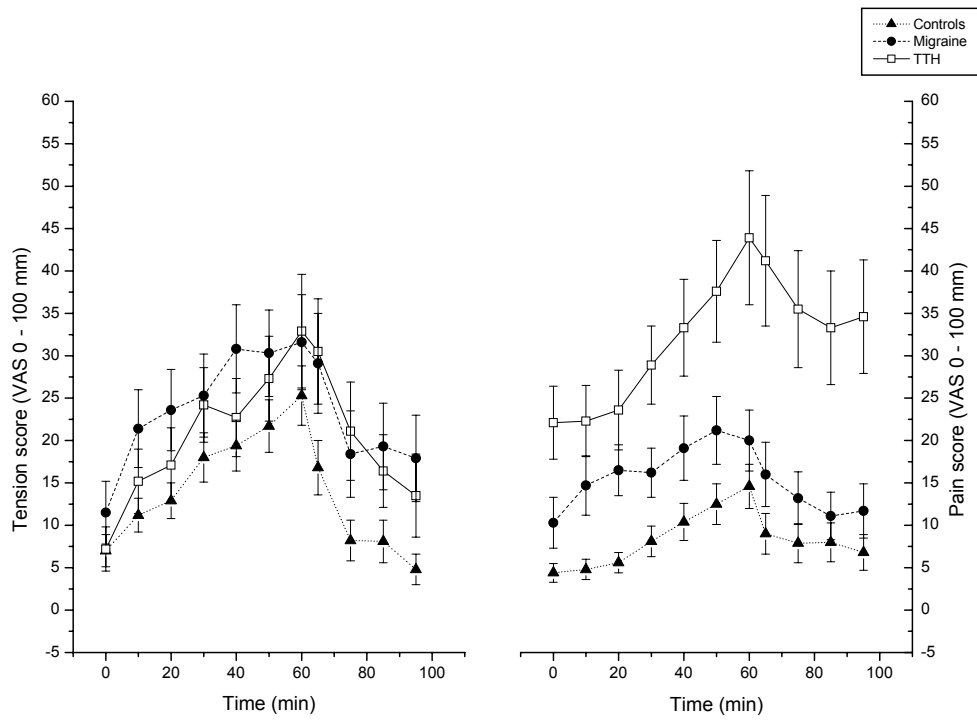
Figure 4: Tension and pain development throughout the stress test and recovery period. Values given as group means (SEM), where maximal reported pain (from the trapezius, splenius, temporalis and frontalis areas, irrespective of side) for each subject was used in the calculations. T=0 – 60: During the cognitive stress test. T= 65 – 95: Relaxation period after the test.

Paper III

Paper III is not included due to copyright.

Dissertations at the Faculty of Medicine, NTNU

1977
1. Knut Joachim Berg: EFFECT OF ACETYLSALICYLIC ACID ON RENAL FUNCTION
2. Karl Erik Viken and Arne Ødegaard: STUDIES ON HUMAN MONOCYTES CULTURED *IN VITRO*

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13. Terje Terjesen: FRACTURE HEALING AN STRESS-PROTECTION AFTER METAL PLATE FIXATION AND EXTERNAL FIXATION.
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<u>Errata</u>

Some variables are presented with wrong numbers in Table 1, page 21 and in paper 2. The correct version of Table 1 is shown below.

Table 1: Background data on subjects included in the study. Pain/tension responses and recoveries are given in group means.

Diagnostic group	Controls (n=44)	Migraine (n=22)	Tension-type headache (n=18)
Gender ratio (F:M)	35:9	20:2	9:9
Mean age (range)	39.0 (19-61)	40.2 (20-60)	34.7 (19-52)
Mean number of years with headache (range)	-	19.9 (7-37)	8.1 (0-32)
Number of subjects with chronic headache (%)	-	4 (18.2)	12 (66.7)
Mean duration (h) of headache attacks (range)*	-	29 (1-72)	34 (8-60)
Number of subjects with aura (%)	-	13 (59.1)	-
Mean general tension (VAS) (range)	25.8 (0-84)	35.1 (1-87)	25.1 (0-65)
Mean EPQ-N score (SD)	7.0 (4.2)	9.1 (4.0)	7.5 (5.1)
Number of subjects who smoke (%)	12 (27.2)	7 (31.8)	2 (11.1)
Body-mass index (SD)	25.0 (3.5)	23.7 (3.5)	24.7 (4.4)

* One migraine patient had some attacks of short duration.
[1] Patients vs. controls, $p \leq 0.05$. [2] Patients vs. controls, $0.05 < p < 0.1$. [3] Migraine vs. TTH $0.05 < p < 0.1$ (Mann-Whitney tests).