Evaluation of offset analgesia in deep muscle pain

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Abstract

Offset analgesia (OA) has been observed as a disproportionately large decrease in pain ratings following a slight intensity decrease in noxious heat stimulation. It is of interest to investigate whether OA is a distinct feature of the heat nociceptive system. The aim of this study was to investigate the existence of OA in deep muscle pain. Seventeen healthy subjects were recruited and a standard heat OA paradigm was applied as a control measurement of the subjects. Temporal summation (TS) and conditioned pain modulation (CPM) were measured in order to assess temporal and spatial pain mechanisms. Pressure OA paradigms were applied using a tourniquet cuff. OA was defined as the minimum pain rating following an incremental decrease in noxious stimulus relative to a time-related pain rating of a constant noxious stimulus. OA was present when applying noxious heat stimulations ($P < 0.001$). The known temporal and spatial pain modulating mechanism TS and CPM showed normal facilitating and inhibitory systems of the subjects. In contrast, OA was absent when applying four different noxious pressure stimuli ($F(3) = 0.227$, $P = 0.750$). Within the limitations of this study the hypothesis of OA as an endogenous analgesic mechanism evoked in deep muscle pain by noxious pressure stimuli cannot be supported.

I. INTRODUCTION

Offset analgesia (OA) was first observed by Grill and Coghill [2002] as a disproportionately large decrease in pain ratings following a slight intensity decrease in noxious heat stimulation. Grill and Coghill [2002] proposed that OA may serve as a temporal contrast enhancement mechanism which amplifies the perception of decreases in temperature stimulus. These findings were supported by a study of Yelle et al. [2008] who found that OA reflects temporal filtering of sensory information as the contrast of dynamic changes in noxious heat stimuli intensity was enhanced.

Studies have investigated the mechanisms involved in OA [Yelle et al., 2008, Martucci et al., 2012a, Derbyshire and Osborn, 2009]. Yelle et al. [2008] showed that OA is partly mediated by central mechanisms, whereas a study by Derbyshire and Osborn [2008] propose that OA could be caused by peripheral mechanisms. Furthermore Niesters et al. [2011] suggested that both peripheral and central mechanisms are involved in the effect of OA. Additionally a study by Martucci et al. [2012a] found that OA is largely opioid-independent and a study conducted by Derbyshire and Osborn [2009] showed that the periaqueductal grey (PAG) and rostroventral medulla (RVM) play an important role in mediating the plasticity of pain during OA.

Complex spatial and temporal phenomena such as OA, graphesthesia and saltation illusion are present in the heat cutaneous nociceptive system [Morch et al., 2010, Grill and Coghill, 2002, Trojan et al., 2006]. However OA has not been observed in noxious pressure stimulations. Previously studies applying the spatial and temporal pain modulation mechanisms conditioned pain modulation (CPM) and temporal summation (TS) have demonstrated that adaptive plastic changes within the human nociceptive system for both cutaneous and deep pain can be induced by stimulus manipulations [Bars et al., 1992, Yarnitsky and Pud, 1994, Derbyshire and Osborn, 2009, Yarnitsky, 2010, Nie et al., 2005]. The mechanisms OA, CPM and TS have shown that noxious stimuli can induce endogenous inhibitory and facilitatory processes, although not necessarily mediated by the

same neural mechanisms [Derbyshire and Osborn, 2009, Nie et al., 2005, Yarnitsky, 2010].

It is unknown whether the same pain mechanism are acting in both cutaneous pain and deep pain. Thus, it is of interest to investigate whether OA is a distinct feature of the cutaneous heat nociceptive system.

The aim of this study was to investigate the existence of OA in deep muscle pain evoked by noxious pressure stimulations.

II. Methods

Subjects

Seventeen healthy subjects (12 males and 5 females), age 24.8 ± 1.4 years (mean ± SD), participated in this study. Written informed consents were provided prior to experimental participation. The subjects acknowledged that they understood the experiment, the methods used, and that they were free to terminate stimulation or withdraw from the study at any time. One subject did not complete the experiment due to scheduling issues and was excluded. The study was approved by the local ethical committee (VEK): N-20120043 and conducted in accordance to the Helsinki Declaration.

Pain ratings

Pain ratings were recorded on a 10 cm long electronic visual analog scale (eVAS) (Aalborg University, Denmark). The extremes (0 and 10) indicated 'no pain' and 'worst imaginable pain'.

Heat stimulations

Thermal stimulations were applied the ventral surface of the dominant forearm 5 cm distal to the elbow joint using a 30x30 mm thermode (rise and fall rate 6°C/s) connected to a Medoc Pathway pain and sensory evaluation system (Medoc, Ramat Yishai, Israel). Two types of stimulation trials were conducted.

Heat OA trials The stimulations consisted of three phases as described by [Grill and Coghill, 2002]: an initial temperature T1 (48°C), an increase to a second temperature T2 (49°C), and a decrease to a third temperature T3 (48°C). T1 and T2 had a duration of 5 s, whereas T3 was prolonged to 20 s.

Heat constant trials The subjects rated their pain intensity during a constant temperature stimulation at T3 (48°C). The heat stimulations were used as controls to investigate whether the subjects were able to elicit heat OA during the original set-up described by [Grill and Coghill, 2002].

Mechanical stimulations

The experimental set-up for mechanical stimulations included a computer-controlled air-compressor (NociTech Aps, Denmark) and a 13 cm wide double chambered pneumatic tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany). The cuff was placed around the shin of the dominate leg at the level of the belly of the gastrocnemius-soleus muscles in accordance with Polianskis et al. [2001]. The air-compressor regulated the compression rate continuously and was controlled by a program written in LabView5 (National Instruments, Austin, Texas, U.S.). The maximal pressure limit was set at 100 kPa, and the inflation could be terminated both by a hand-held release button and from the computer program. During CPM an additional 7.5 cm wide single chambered pneumatic tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was applied.

The pressures applied in TS and pressure OA paradigms were normalized to the subject's pressure pain threshold (PPT = 0 %) and the pressure-pain tolerance (PPTol = 100 %) with pressure A and B defined as 50 % and 75 % respectively. The pressures A1, A2, B1 and B2 corresponded to 30 %, 40 %, 55 % and 65 % respectively.

Pressure pain threshold and pressure pain tolerance The double chambered cuff was inflated at a compression rate of 1 kPa/s. Subjects rated their pain continuously on a eVAS from the first sensation of pain and terminated the stimulation when they reached their pain tolerance. PPT was defined as the first time the VAS exceeded 0 (the pressure value at the transition from a sensation of pressure to a sensation of pain). PPTol was defined as the pressure value at the termination of

pressure inflation.

TEMPORAL SUMMATION TS was assessed by a sequence of ten pressure stimuli (1 s duration and 1 s interstimulus interval) in accordance with Graven-Nielsen and Arendt-Nielsen [2010]. Two series of stimuli were performed, with the double chambered cuff inflated at a pressure intensity corresponding to upper pressure A and B respectively. A constant non-painful pressure of 5 kPa was kept between the individual pressures to ensure that the pressure was applied at the same place for all ten stimuli [Skou et al., 2013]. Pain intensities were rated on the VAS after each stimulus.

CONDITIONED PAIN MODULATION The single chambered cuff was placed around the contralateral upper arm. A noxious pressure corresponding to a VAS 5 rating in a PPT measure of the upper arm was applied as a heterotopic noxious conditioning stimulation for evoking CPM. When a VAS 5 rated pressure was applied to the upper arm a PPT was concurrently performed at the shin of the dominant leg.

PRESSURE STIMULATIONS Measures of the pressure OA paradigm were divided in four conditions each holding a mechanical OA trial and a mechanical constant trial.

MECHANICAL OA TRIALS The method of Grill and Coghill [2002] was modified in order to create pressure trials consisting of three contiguous phases: an initial pressure P1 (lower pressure A1, A2, B1 or B2), an increase to a second pressure P2 (upper pressure A or B), and a decrease to a third pressure P3 (equal to the initial pressure). Each pressure had a duration of 20 s. The mechanical OA trials were used to compare pain ratings to incremental decreases in noxious pressure stimulations with responses evoked by a constant noxious pressure stimulations at the same level.

MECHANICAL CONSTANT TRIALS Constant pressure at intensities of A1, A2, B1 and B2 with a duration of 60 s were applied. Subjects continuously rated the pain intensity. Mechanical trials were separated by 1 min. The four conditions 1:(A,A1), 2:(A,A2), 3:(B,B1) and 4:(B,B2) were presented once per subject in randomized order.

Data and statistical analysis

MinOffset was defined as the minimum VAS score in the P3 interval (gray area in Figure 1). *MinConstant* was defined as the average VAS score for the constant trial at the same time interval as *MinOffset* was detected in the mechanical OA trial (interval between dashed lines in Figure 1). A similar method was used to define *MinHeatOffset* and *MinHeatConstant* of the heat stimulations.

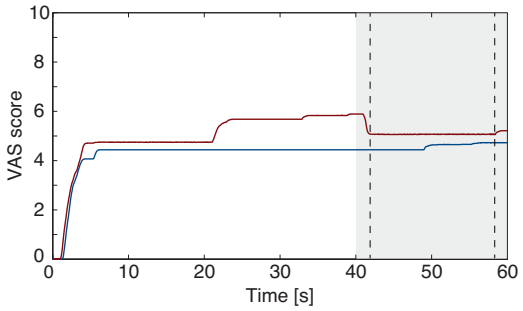

Figure 1: MinOffset was the minimum VAS score in the P3 (gray area). MinConstant was the average VAS score for the constant trial at the same time interval as MinOffset (marked with dashed lines)

A Shapiro-Wilk test was used to determine if the data was normally distributed. Normally distributed data was analyzed using a paired student's t-test and repeated measures analysis of variance (rmANOVA), whereas not normally distributed data was analyzed with a Wilcoxon signed-rank test. In the four conditions a rmANOVA(Pressure,Stimulation) was used to determine if *MinOffset* was significantly different from *MinConstant*. In TS a rmANOVA(Pressure,Stimulation) was used to determine if the average pain ratings corresponding to the initial stimulations were significantly different from maximum pain ratings. Bonferroni post hoc corrections were applied if significant differences were obtained. A Wilcoxon signed-rank test was used to determine if *MinHeatOffset* was statistically different from *MinHeatConstant*. A paired student's t-test determined if PPT pressures at a VAS 7 rating were significantly different from CPM pressures at a VAS 7 rating.

III. Results

HEAT STIMULATIONS The present study found a significant difference in pain ratings between the heat OA trials and the heat constant trials (Wilcoxon signed-rank test: $P < 0.001$) (Figure 4). An average decrease of 4.5 on the VAS was observed in the heat OA trials compared to the heat constant trials.

TEMPORAL SUMMATION The conducted TS experiments at pressure A and B showed no significant difference in pain ratings between the initial and the maximum pain rating (rmANOVA: $F(1) = 3.635$, $P = 0.075$) (Figure 2).

CONDITIONED PAIN MODULATION The CPM experiment showed a statistically significant difference between the pressures corresponding to VAS 7 ratings of PPT and PPT measured with the conditioned heterotopic pain (paired student's t-test: $P < 0.001$). An example is shown in Figure 3

PRESSURE STIMULATIONS From the pressure stimulations a comparison of pain ratings between mechanical constant trials and mechanical OA trials showed no significant difference in the four conditions (rmANOVA: ($F(3) = 0.227$, $P = 0.750$)) (Figure 5 on the next page).

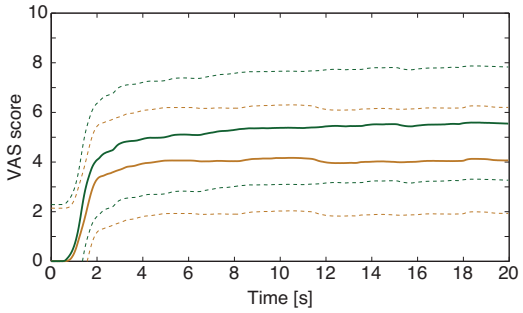

Figure 2: Averaged pain ratings at the two pressures A and B applied during TS. Standard deviations are represented as dashed lines.

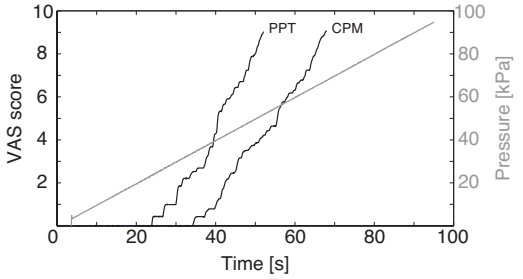

Figure 3: Example of a subject's PPT measurement and a CPM measurement (black) with the continuously increasing pressure (gray) applied during the measurements.

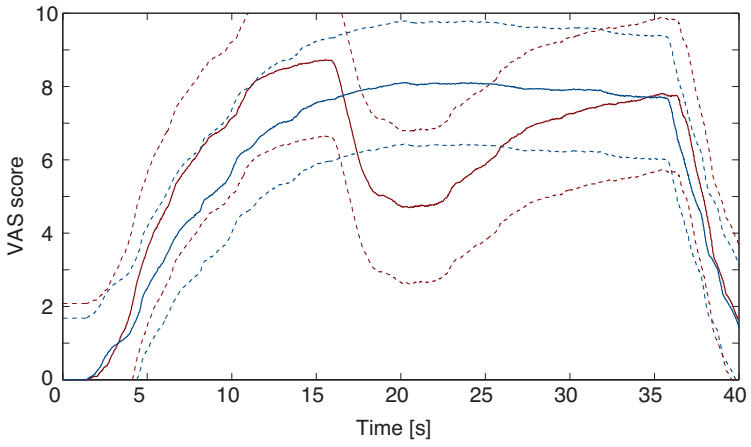

Figure 4: The solid red curve represents the average of the continuous VAS ratings corresponding to the heat OA trials. The solid blue curve represents the average VAS ratings corresponding to the heat OA trials. Standard deviations are represented as dashed lines.

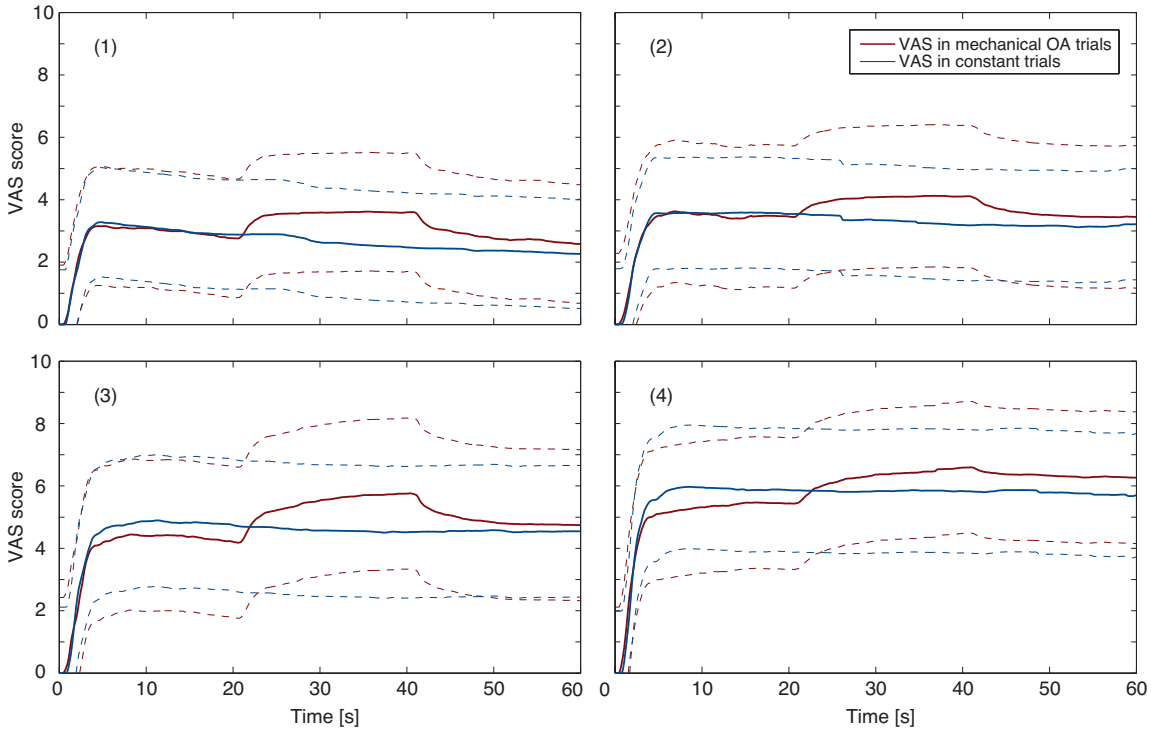

Figure 5: Averaged pain ratings in the mechanical OA trials (solid red) and the mechanical constant trials (solid blue) during the four conditions. Standard deviations are represented as dashed lines.

IV. Discussion

The present study confirmed heat offset analgesia (OA) and temporal summation (TS) and conditioned pain modulation (CPM) as positive controls of the subjects. However, mechanical OA was not found.

Several studies have replicated the findings of Grill and Coghill [2002] by verifying the existence of OA in noxious heat stimulations [Gallez et al., 2005, Derbyshire and Osborn, 2008, Martucci et al., 2012a]. As a control to the main experiment the subjects completed the standard OA paradigm using noxious heat stimulation with three-temperature stimuli of $48-49-48\,^{\circ}C$. Heat OA has been observed at temperature stimuli ranging from $45-46-45\,^{\circ}C$ [Niesters et al., 2011] to $49-50-49\,^{\circ}C$ [Grill and Coghill, 2002]. The control experiment showed a significant OA effect indicating, that subjects do respond to changes in the applied stimuli. This confirmed the existence of heat OA, which further supports the results obtained in previous studies observing the heat OA [Grill and Coghill, 2002, Gallez et al., 2005, Martucci et al., 2012a].

In TS no significant difference between initial and maximum VAS scores was observed. TS is defined as a progressive increment in pain perception during a sequence of stimuli of equal intensity. Enhancement of TS has been linked to central sensitization [Staud et al., 2003, Arendt-Nielsen et al., 2010]. Studies have found that central sensitization is partly activated by glutamate and peptides released by active nociceptors [Kellstein et al., 1990, Marvizon et al., 1997, Bardoni et al., 2004] and that it may result in the pain manifestations (e.g. referred pain) of chronic musculoskeletal disorders [Arendt-Nielsen and Graven-Nielsen, 2003]. TS has furthermore shown to be affected by anxiety [Robinson et al., 2004] and gender [Sarlani et al., 2004]. From a cluster analysis Hastie et al. [2005] found that the cluster associated with increased TS had a significantly higher percentage of females compared to males. This may explain the absence

of TS in the present study as it comprises a majority of males (70 %). Noxious mechanical stimuli applied to fingers and shoulders have additionally induced an enhanced TS in subjects suffering from chronic tension-type headache (CTH) compared to healthy controls [Cathcart et al., 2010]. The absence of TS in the present study may indicate a normal pain facilitation systems and that the subjects were considered not to be central sensitized.

A significant difference is observed between VAS 7 in PPT measurements and VAS 7 in CPM measurements indicating that pain modulation does occur. Studies of CPM have observed an impaired CPM effect in chronic pain conditions such as fibromyalgia [Koseka and Hansson, 1997, Cathcart et al., 2010]. Martel et al. [2013] conducted experiments of the temporal stability of CPM in patients with chronic pain. They observed significant lower effects of CPM in females compared to males. This supports the findings of the present study observing a significant effect of CPM. This may be duo to a majority of males (70 %) enrolled in the experiment. Furthermore Martel et al. [2013] concluded a lack of reproducibility of CPM in males but not in females. The significant effect of CPM in this study furthermore indicates normal pain inhibitory systems and the subjects are considered not to be affected by chronic pain conditions or central sensitization.

Despite verification of OA in noxious heat stimulation and the positive control of TS and CPM it was not possible to confirm OA as an analgesic phenomenon evoked by noxious pressure stimulations. The present study was designed in favor of a potential observation of mechanical OA. To our knowledge, no studies investigating mechanical OA have been conducted. The exact time of interest for observing mechanical OA are therefore unknown. The analysis was constructed to provide OA with the best conditions. This was done by creating algorithms detecting the minimum VAS score from end of P2 to end of P3 and thus yielding the most distinct effect of OA.

Likewise is was unknown at which pressure intensity OA was most pronounced. Studies of heat OA has been conducted at a variety of temperatures; $41-42-41\,^{\circ}C$ [Derbyshire and Osborn, 2009], $45-46-45\,^{\circ}C$ [Niesters et al., 2011, Nilsson et al., 2013], $48-50-48\,^{\circ}C$ [Grill and Coghill, 2002, Marc et al., 2008] and $49-50-49\,^{\circ}C$ [Grill and Coghill, 2002, Yelle et al., 2009, Martucci et al., 2012a]. Additionally the temperatures used to observe heat OA was determined by measures of pain tolerance thresholds (PTTs) and rating on the Gracely intensity scale [Nilsson et al., 2013, Derbyshire and Osborn, 2008]. Accordingly, in this study it was decided to perform the mechanical OA at four different pressures normalized to pressure pain threshold (PPT) and pressure pain tolerance (PPTol) measurements. In this way the experiment has provided the best conditions for OA to be observed. Despite the effort of performing the experiment at different pressures and furthermore applying an algorithm favoring detection of the analgesic responses, this failed. The fact that the experiment design has provided mechanical OA with the best conditions strengthens the absence of OA induce by noxious pressure stimulations.

Grill and Coghill [2002] suggested post-stimulus inhibition as a temporal contrast enhancement mechanism with the purpose of amplifying the perception of stimulus energy decreases. OA, as observed during noxious heat stimulations, was proposed to reflect such a mechanism [Grill and Coghill, 2002]. This was supported by Martucci et al. [2012b] describing OA as a temporal sharpening filter. The contention of a temporal contrast enhancement mechanism in heat may be supported by characteristics of the heat modality. Changes in temperature is not supported by all modalities of the sensory system. Contrary temperature detection, detection of deep muscle pain resulting from e.g strikes or long lasting compressions can be supported by vision and touch. This may give rise to a specific temporal contrast enhancement mechanism of the temperature modality and furthermore it clarifies the absence of this mechanism within the touch modality. However modulations of temporal integration in somatosensory information has been observed [Gabernet et al., 2005].

A pneumatic tourniquet cuff was used to apply noxious pressures in this study. Thus, ischaemia reperfusion injuries may affect the results. Reperfusion injuries occur when blood supply is re-

stored in an ischemic organ or tissue [Carden and Granger, 2000]. The restoration of blood supply when the cuff is deflated can cause micovascular injuries affecting e.g. skeletal muscles and nerves resulting in muscle dysfunction and a burning sensation [Estebe et al., 2011]. If the subjects experience reperfusion pain, this might complicate the results, as it would mask the actual pain relieve that would occur when the pressure is slightly decreased. A study by Tuncali et al. [2006] states that the tourniquet pressure must be 75-100 mmHg greater than the systolic arterial pressure in order to occlude arterial blood. Thus, the P3 pressure applied during OA paradigms must be less than 26-29.3 kPa to cause reperfusion pain. The P3 pressure was in between this interval for 29 % of the subjects. Thus, reperfusion pain may have masked a potential pain relief following a decrease in noxious pressure stimulations.

Another phenomenon which might explain the absence of OA during deep muscle pain is mechano-insensitive nociceptors. C nociceptors can be divided into mechano-responsive and mechano-insensitive nociceptors [Schmidt et al., 1995]. Several studies have shown that mechano-insensitive nociceptors are unresponsive to mechanical stimuli, but become responsive in accordance with inflammation and some do respond to noxious heat stimuli [Schmidt et al., 1995, Schmelz et al., 1997, Weidner et al., 1997]. The mechano-insensitive C nociceptors accounts for approximately 30 % of C nociceptors [Schmidt et al., 1995], and it can be hypothesized that the noxious pressures applied in the present study did not activate these nociceptors. However the noxious heat stimuli used in the present control experiment and the original OA paradigm described by [Grill and Coghill, 2002], might have activated the mechano-insensitive nociceptors causing a different pain sensation. The pain model created by DOMS was applied in order to active these mechano-insensitive C fibers. Nevertheless no significant difference was observed between mechanical OA trials with and without DOMS.

V. Limitations

In a comparison of the present study and previous studies investigating OA two prominent differences in the experimental setup are observed: the stimulation duration and the average pain ratings. In previous studies the standard stimulation duration has been 5-5-20 seconds [Grill and Coghill, 2002, Derbyshire and Osborn, 2008, Gallez et al., 2005] whereas this study on the basis of a pilot study decided to apply stimulations of 20-20-20 seconds. The possibility of adaption may have been minimized by solely prolonging the final interval (5-5-20 seconds). Additionally the average pain rating seem considerably smaller during the experiment applying noxious pressure stimulations compared to those of the control experiment applying noxious heat stimulations. This may influence the magnitude of OA and therefore has to be considered.

VI. Conclusion

Within the limitations of this study the hypothesis of OA as an endogenous analgesic mechanism during noxious pressure stimuli has been rejected. It is suggested that the absent of OA in noxious pressure stimulations is due to lack of temporal contrast enhancement in the submodality of pressure. Additional research is recommended to gain more knowledge of OA as a pain modulating mechanism in pressure stimulation.

VI. References

Arendt-Nielsen, L. and Graven-Nielsen, T. (2003). Central sensitization in fibromyalgia and other musculoskeletal disorders. *Current Pain and Headache Reports*, 7:355–361.

Arendt-Nielsen, L., Nie, H., Laursen, M. B., Laursen, B. S., Madeleine, P., Simonsen, O. H., and Graven-Nielsen, T. (2010). Sensitization in patients with painful knee osteoarthritis. *Pain*, 149:573–581.

Bardoni, R., Torsney, C., Tong, C.-K., Prandin, M., and MacDermott, A. B. (2004). Central sensitization in fibromyalgia and other musculoskeletal disorders. *The Journal of Neuroscience*, 24:2774–2781.

Bars, D. L., Villanueva, L., Bouhassira, D., and Willer, J. C. (1992). Diffuse noxious inhibitory controls (dnic) in animals and in man. pages 55–65.

Carden, D. L. and Granger, D. N. (2000). Pathophysiology of ischaemia-reperfusion injury. *Journal of Pathology*, 190:255–266.

Cathcart, S., Winefield, A. H., Lushington, K., and Rolan, P. (2010). Noxious inhibition of temporal summation is impaired in chronic tension-type headache. *The Journal of Pain*, 50:403–412.

Derbyshire, S. and Osborn, J. (2008). Enhancement of offset analgesia during sequential testing. *European Journal of Pain*, 12:980–989.

Derbyshire, S. W. G. and Osborn, J. (2009). Offset analgesia is mediated by activation in the region of the periaqueductal grey and rostral ventromedial medulla. *NeuroImage*, 47:1002–1006.

Estebe, J.-P., Davies, J. M., and Richebe, P. (2011). The pneumatic tourniquet: mechanical, ischaemia-reperfusion and systemic effects. *Eur J Anaesthesiol*, 28:404–411.

Gabernet, L., Jadhav, S. P., Feldman, D. E., Carandini, M., and Scanziani, M. (2005). Somatosensory integration controlled by dynamic thalamocortical feed-forward inhibition. *Neuron*, 48:315–327.

Gallez, A., Albanese, M.-C., Rainville, P., and Duncan, G. H. (2005). Attenuation of sensory and affective responses to heat pain: Evidence for contralateral mechanisms. *Journal of Neurophysiology*, 94:3509–3515.

Graven-Nielsen, T. and Arendt-Nielsen, L. (2010). Assessment of mechanisms in localized and widespread musculoskeletal pain. *Nature reviews Rheumatology*, 6:599–606.

Grill, J. D. and Coghill, R. C. (2002). Transient analgesia evoked by noxious stimulus offset. *The American Physiological Society*, 87:2205–2208.

Hastie, B. A., III, J. L. R., Robinson, M. E., Glover, T., Campbell, C. M., Staud, R., and Fillingim, R. B. (2005). Cluster analysis of multiple experimental pain modalities. *Pain*, 116:227–237.

Kellstein, D. E., Price, D. D., Hayes, R. L., and Mayer, D. J. (1990). Evidence that substance p selectively modulates c-fiber-evoked discharges of dorsal horn nociceptive neurons. *Brain Research*, 526:291–298.

Koseka, E. and Hansson, P. (1997). Modulatory influences on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (hncs) in fibromyalgia patients and healthy subjects. *The Journal of Pain*, 70:41–51.

Marc, D. Y., June, M. R., and Coghill, R. C. (2008). Offset analgesia: A temporal contrast mechanism for nociceptive information. *Pain*, 134:174–186.

Martel, M. O., Wasan, A. D., and Edwards, R. R. (2013). Sex differences in the stability of conditioned pain modulation (cpm) among patients with chronic pain. *Pain Medicine*, 70:1757–1768.

Martucci, K. T., Eisenach, J. C., Tong, C., and Coghill, R. C. (2012a). Opioid-independent mechanisms supporting offset analgesia and temporal sharpening of nociceptive information. *Pain*, 153:1232–1243.

Martucci, K. T., Yelle, M. D., and Coghill, R. C. (2012b). Differential effects of experimental central sensitization on the time-course and magnitude of offset analgesia. *Pain*, 153:463–472.

Marvizon, J. C., Martinez, V., Grady, E. F., Bunnett, N. W., and Mayer, E. A. (1997). Neurokinin 1 receptor internalization in spinal cord slices induced by dorsal root stimulation is mediated by nmda receptors. *Journal of Neuroscience*, 17:8129–8136.

Morch, C. D., Andersen, O. K., Quevedo, A. S., Arendt-Nielsen, L., and Coghill, R. C. (2010). Exteroceptive aspects of nociception: Insights from graphesthesia and two-point discrimination. *Pain*, 151:42–52.

Nie, H., Arendt-Nielsen, L., Andersen, H., and Graven-Nielsen, T. (2005). Temporal summation of pain evoked by mechanical stimulation in deep and superficial tissue. *The Journal of Pain*, 6:348–355.

Niesters, M., Hoitsma, E., Sarton, E., Aarts, L., and Dahan, A. (2011). Offset analgesia in neuropathic pain patients and effect of treatment with morphine and ketamine. *Anesthesiology*, 5:1063–1071.

Nilsson, M., Piasco, A., Nissen, T., Graversen, C., Gazerani, P., Lucas, M.-F., Dahan, A., Drewes, A., and Brock, C. (2013). Reproducibility of psychophysics and electroencephalography during offset analgesia. *European journal of pain*, pages 1–11.

Polianskis, R., Graven-Nielsen, T., and Arendt-Nielsen, L. (2001). Computer-controlled pneumatic pressure algometry a new technique for quantitative sensory testing. *European Journal of Pain*, 5:267–277.

Robinson, M. E., Wise, E. A., Gagnon, C., Fillingim, R. B., and Price, D. D. (2004). Influences of gender role and anxiety on sex differences in temporal summation of pain. *The Journal of Pain*, 5:77–82.

Sarlani, E., Grace, E. G., Reynolds, M. A., and Greenspan, J. D. (2004). Sex differences in temporal summation of pain and aftersensations following repetitive noxious mechanical stimulation. *Pain*, 109:115–123.

Schmelz, M., Schmidt, R., Bickel, A., Handwerker, H. O., and Torebjork, H. E. (1997). Specific c-receptors for itch in human skin. *The Journal of Neuroscience*, 17:8003–8008.

Schmidt, R., Schmelz, M., Forster, C., Ringkamp, M., Torebjork, E., and Handwerker, H. (1995). Novel classes of responsive and unresponsive c nociceptors in human skin. *The Journal of Neuroscience*, 15:333–341.

Skou, S. T., Graven-Nielsen, T., Lengsoe, L., Simonsen, O., Laursen, M. B., and Arendt-Nielsen, L. (2013). Relating clinical measures of pain with experimentally assessed pain mechanisms in patients with knee osteoarthritis. *Scandinavian Journal of Pain*, 4:111–117.

Staud, R., Cannon, R., Mauderli, A., Robinson, M., Price, D., and Vierck, C. J. (2003). Temporal summation of pain from mechanical stimulation of muscle tissue in normal controls and subjects with fibromyalgia syndrom. *Pain*, 102:87–95.

Trojan, J., Stolle, A. M., Kleinbohl, D., Morch, C. D., Arendt-Nielsen, L., and Holzl, R. (2006). The saltation illusion demonstrates integrative processing of spatiotemporal information in thermoceptive and nociceptive networks. *Exp Brain Res*, 170:88–96.

Tuncali, B., Karci, A., Tuncali, B., Mavioglu, O., Ozkan, M., Bacakoglu, A., Baydur, H., Ekin, A., and Elar, Z. (2006). A new method for estimating arterial occlusion pressure in optimizing pneumatic tourniquet inflation pressure. *Anesthesia and Analgesia*, 102:1752–1757.

Weidner, C., Schmelz, M., Schmidt, R., Hansson, B., Handwerker, H. O., and Torebjork, H. E. (1997). Specific c-receptors for itch in human skin. *The Journal of Neuroscience*, 17:8003–8008.

Yarnitsky, D. (2010). Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. *Current opinion in anaesthesiology*, 23:611–615.

Yarnitsky, D. and Pud, D. (1994). Quantitative sensory testing. *Muscle & nerve*, pages 305–332.

Yelle, M. D., Oshiro, Y., Kraft, R. A., and Coghill, R. C. (2009). Temporal filtering of nociceptive information by dynamic activation of endogenous pain modulatory systems. *The Journal of Neuroscience*, 29:10264–10271.

Yelle, M. D., Rogers, J. M., and Coghill, R. C. (2008). Offset analgesia: A temporal contrast mechanism for nociceptive information. *Pain*, 134:174–186.

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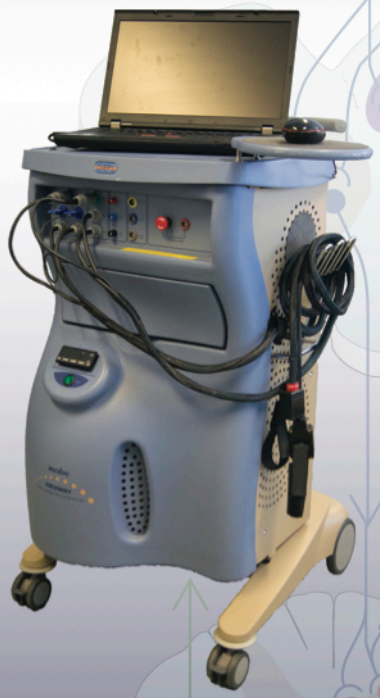

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BREAKING NEW GROUND
AALBORG UNIVERSITY

Aalborg University
Department of Health Science & Technology

"Evaluation of offset analgesia in deep muscle pain"

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Abstract

Offset analgesia (OA) has been observed as a disproportionately large decrease in pain ratings following a slight intensity decrease in noxious heat stimulation. It is of interest to investigate whether OA is a distinct feature of the heat nociceptive system. The aim of this study was to investigate the existence of OA in deep muscle pain. Seventeen healthy subjects were recruited and a standard heat OA paradigm was applied as a control measurement of the subjects. Temporal summation (TS) and conditioned pain modulation (CPM) were measured in order to assess temporal and spatial pain mechanisms. Pressure OA paradigms were applied using a tourniquet cuff. A pain model created by delayed onset muscle soreness (DOMS) was used to evaluate the effect of sensitized nociceptors in mechanical OA. OA was defined as the minimum pain rating following an incremental decrease in noxious stimulus relative to a time-related pain rating of a constant noxious stimulus. OA was present when applying noxious heat stimulations ($P < 0.001$). The well-known temporal and spatial pain modulating mechanisms TS and CPM showed normal facilitating and inhibitory systems of the subjects. In contrast, OA was absent when applying four different noxious pressure stimuli without DOMS ($F(3)= 0.227$, $P = 0.750$) and when subjects were experiencing DOMS ($F(3) = 1.041$, $P = 0.361$). Within the limitations of this study the hypothesis of OA as an endogenous analgesic mechanism evoked in deep muscle pain by noxious pressure stimuli cannot be supported.

Pernille Brøndum

Michael Holt

By signing this document, both members of the project group confirms to have participated in the project work, and thus that they are collectively responsible for the content of this report.

Danish summary - Dansk resumé

Ved varmestimulinger der resultere smerte er fænomentet offset analgesia (OA) blevet observeret som et uforholdsmæssigt stort fald i smertemålinger som følge af en mindre intensitetsreducering i varmestimuleringen [Grill and Coghill, 2002]. Det er interessant at undersøge om OA er en specifik smertemodulerende egenskab opstående under varmestimuleringer. Formålet med dette studie var at undersøge om OA eksisterer i dyb muskelsmerte og om denne mekanisme påvirkes af centrale smertemekanismer og sensibiliserede nociceptorer.

Sytten raske forsøgspersoner (12 mænd og 5 kvinder) i alderen 24-28 år (24.8 ± 1.4 år) deltog i forsøget. Under forsøget blev mekanisk og termisk OA samt temporal summation (TS) og conditioned pain modulation (CPM) målt. Yderligere blev en smertemodel fremkaldt af delayed onset muscle soreness (DOMS) anvendt for at vurdere allerede sensibiliserede nociceptorers effekt på mekanisk OA. Under forsøget blev forsøgspersonerne smerte perception vurderet ved brug af en visuel analog skala (VAS) (0 = ingen smerte, 10 = værst tænkelige smerte). Forsøget blev inddelt i tre sessioner:

Dag 1 Måling af termisk OA
Dag 2 Måling af mekanisk OA, TS og CPM samt inducering af DOMS
Dag 3 Måling af mekanisk OA, TS og CPM under påvirkning af DOMS

OA opstået ved smertefulde termiske stimulationer blev inkluderet, som en kontrolundersøgelse for at verificerer at forsøgspersonerne kan fremkalde termisk OA som beskrevet af Grill and Coghill [2002]. De termiske smerter blev induceret ved brug af en thermode (varmesonde) placeret anteriort på den dominante underarm 5 cm distalt for albueleddet. Tryksmerterne blev induceret ved brug af en trykmanchet placeret omkring m. gastrocnemius-soleus på forsøgspersonernes dominante ben. Temporal summation (TS) og conditioned pain modulation (CPM) blev målt for at vurdere de spatiale og temporale smertemekanismer. TS blev undersøgt ved to trykintensiteter med stimuleringer bestående af ti impulser (1 s varighed, 1 s interstimulus varighed). For at undersøge CPM blev der foretaget målinger af forsøgspersonernes smertetærskel og -tolerance sideløbende med påførelsen af tryksmerte omkring overarmen. Forsøget med mekanisk OA blev inddelt i fire delforsøg udført med forskellige tryk normaliseret til forsøgspersonernes smertetærskel og -tolerance.

Termisk og mekanisk OA blev defineret som det maksimale fald i smertemålingerne efter en mindre reducering i stimulationsintensitet sammenlignet med en tidsrelateret smertemåling under en konstant smerte stimulation. Ud fra målingerne af TS blev der bestemt to parametrer: en initierende smertemåling og en maksimal smertemåling. Størrelsen af TS blev defineret som smerteforøgelsen mellem disse parametre. Effekten af CPM blev fundet ved at sammenlige CPM-målingerne med målinger af smertetærsklen. Sammenligningen blev foretaget ved en trykintensitet svarende til en smertemåling på 7.0.

OA blev observeret ved smertefulde termiske stimuleringer ($P < 0{,}001$). De kendte spatiale og temporale smertemodulerende mekanismer CMP og TS viste at forsøgspersoner havde normale faciliterende og hæmmende systemer. I modsætning hertil kunne OA ikke fremkaldes under anvendelsen af fire forskellige trykstimulationer hverken med DOMS ($F(3) = 1.041$, $P = 0.361$) eller uden inductionen af DOMS ($F\ (3) = 0{,}227$; $P = 0{,}750$).

Inden for begrænsningerne af dette studie kan hypotesen om OA som en endogen smertelindrende mekanisme fremkaldt i dyb muskelsmerter ikke understøttes.

Preface and reading instructions

Preface

This thesis is composed by group 1073 as a completion of the master's education in Biomedical Engineering and Informatics at the Department of Health Science and Technology at Aalborg University. The thesis was conducted and written from February 3rd 2014 to June 3rd 2014.

The intended target group is primarily researchers with interest in the pain phenomenon Offset Analgesia (OA). The secondary target group is supervisors, censor and fellow students. It can be an advantage to have knowledge about pain modulating mechanisms and OA.

Reading instructions

This report functions as a supplement to the article *Evaluation of offset analgesia in deep muscle pain*. The report is not meant to be read independently from the article. The report includes: background information, a more profound description of the experiment, data analysis, results and discussion. In addition an appendix has been added with the chapters Pilot study and Experiment protocol and cross-references to these chapters were made throughout the report.

References to publications within the article and the report are given in two ways. References which are stated before a period refer to the previous sentence whereas references stated after a period refer to the previous section. All references are written in accordance with the Harvard method. The author's surname(s) and the publication year are stated in squared brackets: E.g. ['surname(s)', 'publication year']. For references with more than two authors the term et al. is used: E.g. ['first author's surname' et al., 'publication year'].

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1

Introduction

Offset Analgesia (OA) was first observed by [Grill and Coghill, 2002] as a disproportionately large decrease in pain ratings following a slight intensity decrease in noxious heat stimulation.[Grill and Coghill, 2002] proposed that OA may serve as a temporal contrast enhancement mechanism which amplifies the perception of decreases in temperature stimulus. These findings were supported by a study of [Yelle et al., 2008a] who found that OA reflect temporal filtering of sensory information as the contrast of dynamic decreases in noxious heat stimulus intensity were enhanced.

Studies have investigated the mechanisms involved in OA [Derbyshire and Osborn, 2009, Martucci et al., 2012b, Yelle et al., 2008a]. Yelle et al. [2008a] showed that OA is partly mediated by central mechanisms, whereas a study by Derbyshire and Osborn [2008] propose that OA could be caused by peripheral mechanism. Furthermore Niesters et al. [2011] suggested that both peripheral and central mechanism are involved in effect of OA. Additionally a study by Martucci et al. [2012b] found that OA are largely opioid-independent and a study conducted by Derbyshire and Osborn [2009] showed that the periaqueductal grey (PAG) and rostroventral medulla (RVM) play an important role in mediating the plasticity of pain during OA.

Complex spatial and temporal phenomena such as OA, graphesthesia (Identification of numbers 'written' on the skin) and saltation illusion (perceived spatial distortion of stimuli presented in spatio-temporal patterns) are present in the heat cutaneous nociceptive system [Grill and Coghill, 2002, Morch et al., 2010, Trojan et al., 2006].

Previously studies applying the spatial and temporal pain modulation mechanisms conditioned pain modulation (CPM) and temporal summation (TS) have demonstrated that stimulus manipulations can induce adaptive plastic changes in the human nociceptive system for both cutaneous and deep pain [Bars et al., 1992, Derbyshire and Osborn, 2009, Nie et al., 2005, Yarnitsky and Pud, 2004, Yarnitsky et al., 2010]. The mechanisms OA, CPM and TS have shown that noxious stimuli can produce endogenous inhibitory and facilitatory processes, although not necessarily mediated by the same neural mechanisms [Derbyshire and Osborn, 2009, Grill and Coghill, 2002, Nie et al., 2005, Yarnitsky, 2010].

A study by Niesters et al. [2011] showed that OA was absent in neuropathic patients. Thus far, OA has not been investigated in other pain conditions. Delayed onset muscle soreness (DOMS) is muscle soreness combined with tenderness and stiffness and this mechanism can be used as a pain model to sensitize nociceptors [Nosaka, 2008].

It is unknown whether the same pain mechanism are acting in both cutaneous pain and deep pain. Thus, it is interesting to investigate whether OA is a distinct feature of the cutaneous heat nociceptive system. The aims of this study was to investigate the existence of OA in deep muscle pain evoked by noxious pressure stimulations. Furthermore to evaluated whether OA is affected by central acting mechanisms and sensitized nociceptors.

2

Pain

Pain is a complex sensory modality which is essential for survival and it is described as an unpleasant sensory experience associated with discomfort [Silbernagl and Despopoulos, 2009]. It functions as a protective mechanism which provides information about abnormalities in the body or stimuli that have potential to cause tissue damage [Kopf and Patel, 2010, Silbernagl and Despopoulos, 2009]. Painful stimuli activates specialized receptors called nociceptors, and activation of these receptors have the potential to cause pain. Nociception is the reception and central processing of noxious stimuli by the central nervous system (CNS), whereas pain is the perception of the subjective sensation. [Kopf and Patel, 2010, Siegel and Sapru, 2011]

2.1 Neurophysiology of pain

Nociceptors are free nerve endings that transform sensory stimuli into nerve impulses, which the brain interprets in order to produce a sensation of pain [Kopf and Patel, 2010, Silbernagl and Despopoulos, 2009]. Nociceptors are classified according to their nerve fibers. Most of these fibers are unmyelinated, slowly conducting C fibers (< 1 m/s) or faster, lightly myelinated Aδ-fibers (5–30 m/s) [Silbernagl and Despopoulos, 2009]. Nociceptors are furthermore divided into three receptor types according to their functionality: Mechanical receptors, thermal receptors and mechanothermal and polymodal receptors [Siegel and Sapru, 2011]. C fibers are polymodal receptors, which are activated by chemical, mechanical and thermal stimuli and they carry the sensation of slow dull and long lasting pain to CNS. A-delta fibers are unimodal receptors that respond to mechanical, thermal and mechanothermal stimuli, and these fibers carry information of fast, sharp and localized pain to CNS. [Kopf and Patel, 2010, Siegel and Sapru, 2011, Silbernagl and Despopoulos, 2009] The nerve fibers of both A-delta and C fibers enter the spinal cord at the apex of the dorsal horn. Here they branch and then ascend or descend two or three segments before entering the dorsal horn. [Siegel and Sapru, 2011]

Pain pathways

Ascending pathways mediating pain

The two major ascending nerve pathways involved in transmitting pain signals from the body and the head/face to higher centers are the neospinothalamic tract and the trigeminal tract [Siegel and Sapru, 2011].

The soma of sensory neurons responsible for mediating pain are located in the dorsal root ganglia, these neurons are known as first-order neurons. The nerve ending of the peripheral axons of first-order neurons are represented by nociceptors and their central axons reach the dorsal horn, from which they branch into ascending and descending nerves innervating specific areas, forming the dorsolateral tract of Lissauer. In Lissauers tract Aδ and C fibers ascend or descend one–two spinal segments before entering the spinal gray matter and then synapse on neurons located in laminae I and II. The sensory information is then carried to second-order neurons located in laminae IV-VI. [Siegel and Sapru, 2011] The neospinothalamic tract arise from the second-order neurons in laminae IV-VI. The axons of these second-order neurons, which mediate noxious signals, cross the spinal midline to the contralateral side in the anteriorlatoral funiculus,

where they form the neospinothalamic tract (Figure 2.1 a). The neospinothalamic tract ascend centrally through medulla, pons, and the midbrain before projecting upon nerve cells located in specific areas of thalamus (ventral posterolateral nucleus and posterior nuclei). From the specific areas of thalamus, the axons of the thalamic nerve cells project to the primary sensory cortex. [Kopf and Patel, 2010, Siegel and Sapru, 2011]

Nerve fibers originating from the trigeminal ganglion and cranial nuclei VII, IX and X mediate the nociceptive information from the face and head area through the trigeminal tract (Figure 2.1 b). These nerve fibers enter the brain stem, where they cross the neural midline to project upon nerve cells of the contralateral side of thalamus. The axons of the thalamic nerve cells then project to the cerebral cortex and the sensory area of the face, obit, nose and mouth. [Kopf and Patel, 2010, Siegel and Sapru, 2011, Silbernagl and Despopoulos, 2009]

The thalamic areas which receives pain information from the neospinothalamic tract and the trigeminal tract also receives somatosensory information [Silbernagl and Despopoulos, 2009]. By having both nociceptive and somatosensory information projecting on the same cortical area, pain can be described as a 'localized painful experience' according to the intensity and location. Knowledge about the complexity of pain pathways, can contribute to localizes the origin of pain. [Kopf and Patel, 2010]

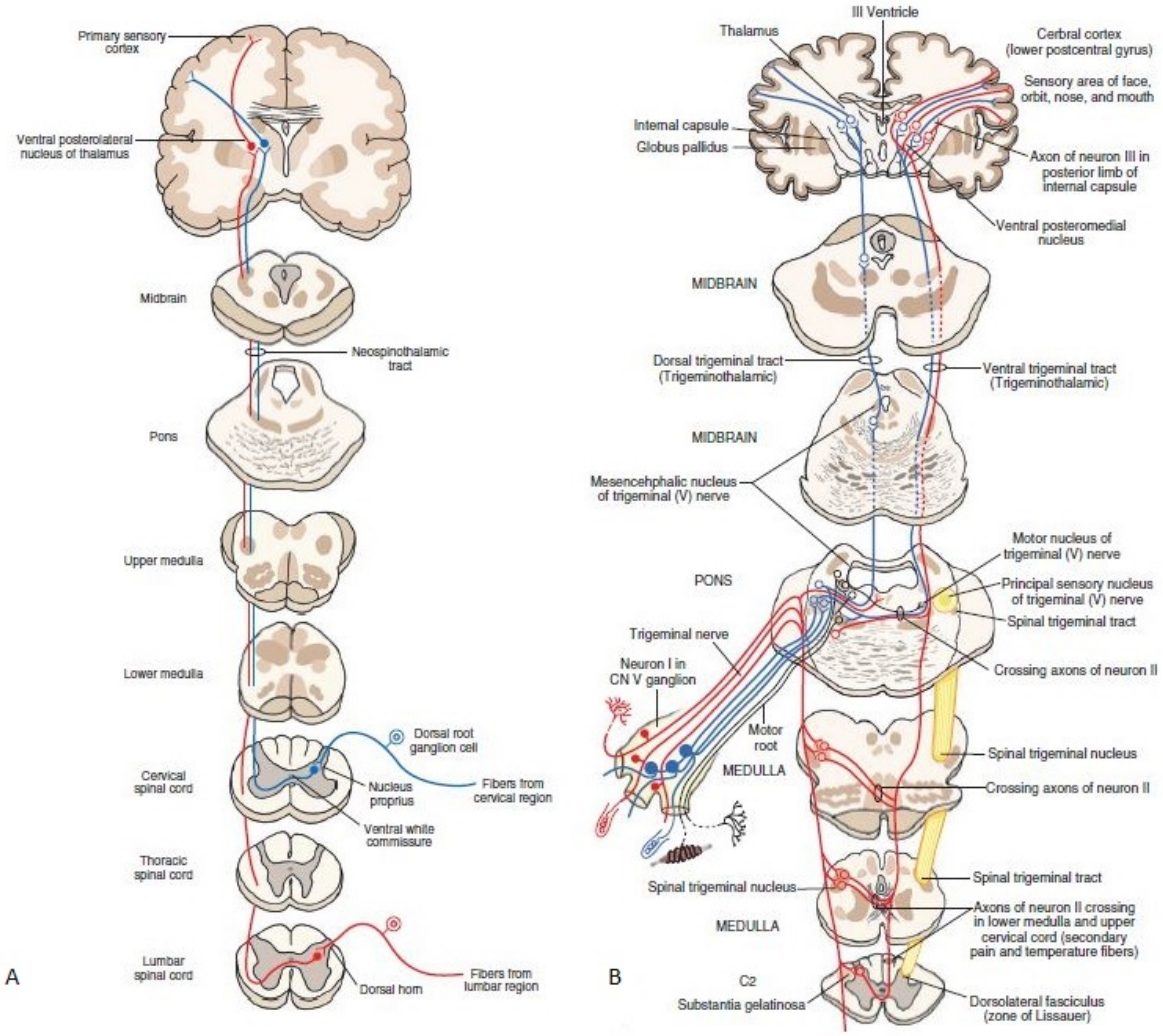

Figure 2.1: *A) The neospinothalamic tract conveying nociceptive information from the body B) The trigeminal tract conveying nociceptive information from the face and head. [Siegel and Sapru, 2011]*

Descending pathways modulating pain

The sensation of pain can be modulated by descending pathways. The pathway from the periaqueductal gray: The neurons which are located in periaqueductal gray matter (PAG) of the midbrain project on neurons located in nucleus raphe magnus of the midline medulla. It has been shown that electrical stimulation of PAG suppress the activity of nociceptive mechanism, and it is therefore proposed that stimulation of PAG excites neurons in nucleus raphe magnus, which modulate the sensation of pain. [Siegel and Sapru, 2011]

The pathway from nucleus raphe magnus: The neurons in nucleus raphe are serotonergic (a synapse which uses serotonin as its neurotransmitter) and their axons descend through several levels of the spinal cord and form synapses on enkephalin (an endogenous opioid peptide) interneurons located in the spinal cord. Stimulation of the descending neurons in the nucleus raphe excites the enkephalinergic interneurons and when enkephalins are released from these interneurons, neurotransmitters from central processes of nociceptive neurons in the dorsal root ganglion are inhibited. The second-order dorsal horn neurons from which the neospinothalamic tract arises are also inhibited by the release of enkephalin, and it produces an analgesic effect [Siegel and Sapru, 2011].

The noradrenergic pathway: Noradrenergic locus ceruleus neurons located in the upper pons have axons descending through medulla to the dorsal horn. These neurons do also form synapses with the enkephalinergic interneurons and provide the same analgesic effect, by the release of enkephalin [Siegel and Sapru, 2011].

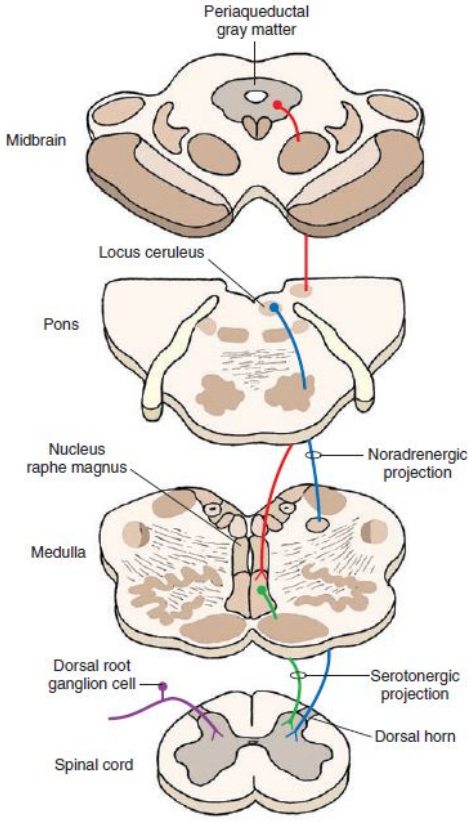

Figure 2.2: *The pathway from the periaqueductal gray matter is illustrated in red, the pathway from Nucleu Raphe Magnus is illustrated in blue and the Noradrenergic pathway is illustrated in green. [Siegel and Sapru, 2011]*

Neurotransmitters involved in pain pathways

Glutamate and substance P are thought to be the neurotransmitter released at first-order nociceptive neurons in the dorsal horn. These neurotransmitters excite second-order spinothalamic dorsal horn neurons, whose axons cross to the contralateral side and form the ascending neospinothalamic tract. First-order nociceptive dorsal root ganglion neurons form presynaptic opiate receptors, whereas second-order spinothalamic neurons form postsynaptic opiate receptors. The dorsal horn enkephalinergic interneurons form axo-axonal synapses at the central processes's terminals of first-order nociceptive dorsal root ganglion neurons and axo-dendritic synapses on dendrites of second-order spinothalamic neurons. When the enkephalinergic interneurons are activated by the projection of serotonin-containing neurons in nucleus raphe magnus and noradrenergic locus ceruleus, enkephalin is released and binds to opiate receptors of the central processes's terminals of nociceptive dorsal root ganglion neurons. Thus, Ca^+ entry into the terminal is reduced, and the release of substance P and glutamate is decreased. Postsynaptic opiate receptors on the dendrites of second-order spinothalamic neurons are also activated by the release of enkephalin, as they are hyperpolarized by an increase in the K^+ conductance and inhibited. Thus, the release of enkephalin, by activation of descending serotonergic projections to the dorsal horn, attenuate the effects of nociceptive stimuli. [Siegel and Sapru, 2011]

2.2 Neurophysiology of muscle pain

Most nociceptors appear to have a common physiological basis, but there are some differences between skeletal muscle nociceptors and other nociceptors [Nosaka, 2008].

Nociceptors in muscles

The sensation of muscle pain differs from the sensation of cutaneous pain, as pain associated with muscle lesions is characterized as aching and cramping pain, whereas cutaneous pain is described as sharp, stabbing or burning sensation [Nosaka, 2008]. The nociceptive A-delta fibers in muscles respond to muscle stretch, contractions and innocuous pressure and they are sensitized by thermal and chemical stimuli. The C fibers are sensitized by chemical stimuli and they respond to thermal stimuli, ischemia and hypoxia. By stimulating muscle C fibers a dull, aching and cramping pain is elicited. It is thought that the sensation of pain arising from muscles are primary mediated by C fibers and secondarily by A-delta fibers [Nosaka, 2008]. Nociceptors in skeletal muscles are located along the walls of the arterioles and in the surrounding connective tissue, and there are no nociceptors in the muscle plasma membrane. Muscle fibers can be damaged without the sensation of pain if the nociceptors are not located in the affected area. Thus, the sensation of muscle pain is activated by chemical changes in the surrounding tissue or stimulation of the fascia, rather than actual muscle cell damage. [Nosaka, 2008]

Pain pathways in muscle pain

There are two spinothalamic pathways; the neospinothalamic (lateral spinothalamic) tract and the paleospinothalamic (anterior spinothalamic) tract. Pain signals from A-delta fibers are primarily transmitted through the neospinothalamic tract and the sensation appears to be sharp and fast. In contrast the paleospinothalamic tract, which terminates widely in the brain stem and thalamus transmits pain signals from C fibers and the pain sensation is dull and aching. The spinothalamic tract terminates at thalamus and pain signals from both A-delta and C fibers are then transferred to the primary somatosensory cortex. [Nosaka, 2008]

3

Pain mechanisms

3.1 Central and peripheral sensitization

Painful stimuli may result in tissue damage. The phenomenon hyperalgesia is defined as an increased pain from a stimulus that normally provokes pain [IASP, 2012]. This can be caused by peripheral sensitization [Purves et al., 2012]. Tissue damage results in the release of a variety of substances in the affected area purposed to protect the area, promote healing and guard against infection. The substances (neurotransmitters and peptides) comprise an 'inflammatory soup' which, by interaction with nociceptors, causes peripheral sensitization (increased pain sensitivity). The majority of the non-neural cells present in the inflamed area causes a direct interaction with the nociceptors and thereby augmenting the response of the nociceptive fibers. Responses of the TRPV1 heat receptor is augmented by interaction with cellular proteins, lipid metabolites, NGF and bradykinin. Additionally the depolarization threshold of specific sodium channels within the nociceptors is reduced in the presence of prostaglandins. [Purves et al., 2012]

Activity in nociceptors may also create central sensitization. This phenomenon is found in the spinal cord and causes an increased excitability. An increasing excitability of nociceptive C fibers is known as the wind-up effect in which C fibers are firing repetitively. The dorsal horn neurons are additionally sensitized in the activation of protein kinases. [Kandel et al., 2013] Thus previous insufficient subthreshold afferent signals now generate action potentials resulting in an increased pain sensitivity. These physiological alterations are due to central sensitization. [Purves et al., 2012]

3.2 Temporal summation

Animal and human studies have found that the generation of pain depends on the number of stimulated afferent nerve fibers, the stimulation frequency and stimulation intensity [Arendt-Nielsen and Yarnitsky, 2009, Graven-Nielsen and Arendt-Nielsen, 2008; 2010]. It has been shown that temporal summation (TS) of painful stimuli increases the magnitude of pain along series of stimuli and that it is the psychophysical correlation to wind-up [Price et al., 1994, Ren, 1994]. TS is a central mechanism, which is defined as a progressive increase in pain perception occurring when unchanged repeated stimuli become increasingly painful, whereas wind-up describes the progressive increase in number of action potentials evoked by each stimulus from the spinal cord second-order neurons as a response to the repeated stimulation of the peripheral C or A-delta fibers [Arendt-Nielsen and Graven-Nielsen, 2004, Arendt-Nielsen and Petersen-Felix, 1995, Graven-Nielsen and Arendt-Nielsen, 2010]. Wind-up is generated immediately and occurs when the stimulation rate is below 0.3 Hz [Arendt-Nielsen et al., 1994]. The TS can be described as the difference in the pain sensation between the first and last stimulus in a series of stimuli [Graven-Nielsen and Arendt-Nielsen, 2010, Yarnitsky and Pud, 2004]. TS of pain induced by heat, mechanical and electrical stimuli is a well documented phenomenon in normal human skin and deep tissue [Graven-Nielsen et al., 2000, Koltzenburg and Handwerker, 1994, Lemming et al., 2012, Nielsen and Arendt-Nielsen, 1998]. It has been shown that stimulus close to the pain threshold can give rise to pain, if the stimulus is repeated e.g. 10 times, within a suitable stimulation rate. Furthermore it has been shown that painful stimulation can be perceived

stronger when repeated, and by central sensitization the summation will be amplified [Arendt-Nielsen and Yarnitsky, 2009, Arendt-Nielsen et al., 2010, Graven-Nielsen and Arendt-Nielsen, 2010].

3.3 Spatial summation

Spatial summation is a central mechanism which refers to an increase in pain perception intensity when the stimulation area is increased [Graven-Nielsen et al., 2012, Staud et al., 2007]. It is the nervous system's ability to integrate nociceptive information from larger areas of the body, and it is essential for pain detection, pain intensity coding and pain quality identification [Defrin et al., 2003]. It is suggested that spatial summation depends on the spatial resolution of the nociceptive system, as the number of innervated nociceptors following cutaneous and deep tissue damage is increased. Thus, a given noxious stimulus activates a larger number of nociceptors than normal, which leads to a decrease in pain threshold and an increase in pain perception [Defrin et al., 2003].

3.4 Conditioned pain modulation/Diffuse noxious inhibitory control

Throughout the literature the term diffuse noxious inhibitory control (DNIC) is used to describe the paradigm in which a noxious stimulus is used as a conditioned stimulus to induce a decrease in pain perception by another stimulus [Yarnitsky et al., 2010]. When a noxious stimulus is applied to one part of the body it inhibits the dorsal horn nociceptive neurons in the spinal segments which innervates distant body parts [McMahon and Koltzenburg, 2006]. It has been shown that primate spinothalamic tract neurons which are excited by noxious stimuli delivered to one foot can be inhibited by noxious stimulation applied to either the face or the contralateral foot [Gerhart et al., 1981]. The theoretical basis for the inhibitory control activated by noxious stimuli is based on the DNIC hypothesis. This hypothesis states that noxious stimuli activate a surrounding inhibition that sharpens the contrast between the stimulated area and the adjacent areas. This contrast has a net enhancing effect on the perceived pain intensity. However outside the stimulated area a net analgesic effect will arise. [Le Bars, 2002] DNIC is triggered by peripheral A-delta and C fibers, involved brain structures in the caudal-most part of the medulla including the subnucleus reticularis dorsalis (SRD) and it is mediated by descending pathways in the dorsolateral funiculi [Le Bars, 2002]. The term DNIC was originally used in animal based research to describe a specific lower brainstem mediated inhibitory mechanism. Human psychophysical research, has indiscriminately adopted this term but specific mechanisms cannot be distinguished, such as the net effect of complex facilitatory and inhibitory mechanisms of pain processing. It was therefore decided in 2010 that the term conditioned pain modulation (CPM) should be used to describe the psychophysical paradigm where a conditioned stimulus is used to affect a test stimulus in human research, instead of DNIC. [Yarnitsky et al., 2010]

3.5 Offset analgesia

The analgesic phenomenon offset analgesia (OA) was named by Grill and Coghill [2002] in a demonstration of a potential analgesia evoked by decreases in noxious stimulus temperatures. Their experiments consisted of three experimental trials in which the subjects rated the temperature pain intensity. The trials featured incremental increasing and decreasing noxious stimulus temperatures with a 5 s duration resulting in an initial painful stimulus, followed by an increase and a decrease. Additionally control trials and trials at a constant temperature were performed. They demonstrated that a decrease in noxious stimulus temperature triggers a potential analgesia and showed that it is distinct from previously observed adaptation and/or primary afferent fatigue in noxious stimulation. [Grill and Coghill, 2002]. Grill and Coghill [2002] also concluded

that OA has indications of being an active process and that central inhibitory mechanisms are involved.

Pain attenuation has been found in studies where heat stimuli are presented over longer time periods. In a study of Gallez et al. [2005] subjects participated in five sessions spaced between one and six days apart in which they were presented to noxious heat stimuli. The observed attenuation to the heat pain perception was also found to be highly body side specific. It it suggested that the potential underlying plasticity occurred in brain areas displaying contralateral bias or a strict pattern of contralateral activation [Gallez et al., 2005].

Offset analgesia has been confirmed in more recent studies as well [Martucci et al., 2012b]. Both within-session adaption and OA was observed in a study of Derbyshire and Osborn [2008]. Derbyshire and Osborn [2008] extended the studies of Grill and Coghill [2002] and Gallez et al. [2005] in order to investigate OA over repeated experimental sessions. This study showed that the effects of OA was exaggerated by the concurrent adaptation and that the adaption, in high pain trials, was significantly smaller than the effects of OA.

In studies using noxious heat stimuli OA showed to increase over time when several sessions were performed. In the study of Derbyshire and Osborn [2008] this was explained with a conditioned analgesic response. The predictable stimuli of the study resulted in less threatening future stimulations. The subjects may learn that the stimuli creates no tissue damage and the temperature decrease may even serve as a reward to the subject. The cortical structures insula and anterior cingulate cortex may be mediating the noxious stimuli attenuation that is observed across days while subcortical structures such as the rostroventral medulla (RVM) and periaqueductal grey (PAG) may be mediating OA. Another possible explanation is that OA responses are caused by peripheral changes. [Derbyshire and Osborn, 2008]

Experiments of Reynolds [1969] have shown how electrical stimulation of the PAG in rats made surgical advancement possible without further anesthesia. Additionally anatomical studies have shown how analgesia rely on transmissions of the RVM in the insula [Gebhart et al., 1983, Sandkuhler and Gebhart, 1984]. A more recent fMRI study measured brain activity during an OA procedure [Derbyshire and Osborn, 2009]. The findings provided significant evidence that the inhibitory mechanism is mediated by activity in the PAG/RVM regions of the brain. Furthermore the insula and related regions normally active during pain processing were shown to have a reduced activity during OA. It is generally accepted that this inhibition is linked to projections from the PAG to the RVM which projects along the dorsolateral funiculus to the dorsal horn, at which nociceptive transmission is inhibited [Vanegasa and Schaible, 2004]. The RVM is described to hold so-called on-cells and off-cells which either facilitate or inhibit nociceptive transmission. These are both triggered during the nociceptive transmission and this balance may be altered towards inhibition when a highly noxious stimulus changes to a less noxious stimulus. [Fields, 2004] A state of chronic pain may partly be caused by a failure of these descending inhibitory activities [Derbyshire and Osborn, 2009].

The temporal transformation occurring during OA may serve as a temporal sharpening filter. The inhibitory mechanism of OA increases the temporal contrast of the stimulus and thereby serves as a enhancement in escape behaviors. [Grill and Coghill, 2002, Yelle et al., 2008b] In a study of Yelle et al. [2008b] this temporal filtering properties of OA was tested by altering the stimulus fall rates during a OA procedure using heat pain. The results showed a more rapid rate of decrease in perceived pain compared to that predicted from the fall rate in stimulus. Furthermore the perceived rate of change in stimulus intensity was found to be independent from the actual stimulus fall rate. Based on these two findings Yelle et al. [2008b] described the observed temporal contrast enhancement as an edge enhancement.

3.6 Delayed onset muscle soreness

Delayed onset muscle soreness (DOMS) is muscle soreness combined with tenderness and stiffness, which may last for several days post-exercise. DOMS arises due to unaccustomed or severe exercise consisting of repeated eccentric contractions (force lengthening), which result in muscle damage. The delay in DOMS varies among individuals but the soreness normally increases in intensity within the first 24 hours and peaks before 72 hours post-exercise before it disappears after five to seven days. The sensation of pain is typically perceived when the affected muscle is imposed by mechanical stimulation such as pressure, stretching or contraction, whereas no or little discomfort is present during rest [Nosaka, 2008].

Mechanism of DOMS

The mechanism of DOMS is not fully understood, and a number of theories have been proposed in order to explain it; lactic acid, muscle spasm, muscle damage, connective tissue damage and inflammation [Cheung et al., 2003]. The pain sensation is primarily thought to be a result of mechanical induced muscle damage, as theories pertaining to the build up of lactic acid in blood and muscle spasms have been largely rejected [Cleak and Eston, 1992, Miles and Clarkson, 1994]. DOMS was first described by Hough [1902], who concluded that DOMS is fundamentally the result of ruptures within the muscle. Although ruptures of muscle fibers are not associated with DOMS, microscopic lesions of myofilaments and especially the Z-disc of the muscle sarcomere have been reported to be the prime factor contributing to DOMS [Cleak and Eston, 1992, Friden et al., 1984, Yu and Thornell, 2002]. The damage theory proposed by Hough [1902] is still valid with some modifications, as it is most likely that muscle or connective tissue damage, and subsequently inflammatory responses are associated with DOMS [Cheung et al., 2003].

4

Measurements of pain

4.1 Visual analog scale

The visual analog scale (VAS) is one of the most commonly used measures of pain intensity in clinical trials and pain research, and the reliability is generally good [Gift, 1989, Jensen et al., 2003, McMahon and Koltzenburg, 2006, Sindhu and Shechtman, 2011]. The VAS is often a 10 cm long scale, which contains two descriptors representing the extremes of pain intensity (e.g. zero corresponding to 'no pain' and ten corresponding to 'worst imaginable pain') [Price et al., 1994]. Patients or test subjects rate their pain by marking/moving the slider to a point somewhere on the scale that indicates their pain level and the VAS score is defined as the distance from zero to the marked point (e.g. VAS 4 = 4 cm from zero). [Jensen et al., 2003, Sindhu and Shechtman, 2011] An electronic VAS (eVAS) has the advantage that it provides a continuous description of the pain and changes in pain intensity over time, which is not accessible with non-electronic scales [Graven-Nielsen et al., 1997, McMahon and Koltzenburg, 2006].

4.2 Pressure pain threshold

A pain threshold is by the International Association for the Study of Pain (IASP) defined as *"the minimum intensity of a stimulus that is perceived as painful"* [IASP, 2012]. Pressure pain threshold (PPT) is a.i. used in studies of mechanoreceptors [Graven-Nielsen et al., 2004], pain conditions [Jespersen et al., 2013] and central sensitization [Coronado et al., 2014].

4.3 Pain tolerence level

The pain tolerance level is by IASP defined as *"the maximum intensity of a pain-producing stimuli that a subject is willing to accept in a given situation"* [IASP, 2012]. Pressure pain tolerance (PPTol) has a.i. been used in studies of Alzheimer disease [Jensen-Dahm et al., 2014] and transcutaneous electrical nerve stimulation [,õtak Karakaya et al., 2014].

5

Experiment

The aim of this study was to investigate the existence of offset analgesia (OA) when applying noxious pressure stimulations in a standard OA paradigm. In order to support the findings of the experiment and a possible existence of OA in deep muscle pain, a pain model, control paradigms including spatial and temporal pain modulating mechanisms and a control experiment using noxious heat stimuli were introduced. The framework of the experiment consisted of three main parts:

Day 1 The subject completes standard OA paradigms using noxious heat stimulations.
Day 2 The subject completes OA paradigms induced by noxious pressure stimulations and control paradigms with central acting pain mechanisms. Furthermore the subject performs calf-raise exercises in order to induce delayed onset muscle soreness (DOMS) as a pain model.
Day 3 The subject repeats the OA paradigms and the control paradigms with DOMS.

In standard measurements of OA intensity-equal heat stimulations were used. During this experiment the pressure were normalized to the individual subject. The OA effect was measured by comparing pain ratings of pressure trials applying constant stimulations with trials applying incremental increasing and decreasing stimulations. The applied pain model was created by having subjects performing calf-raise exercises in order to induce DOMS in the gastrocnemius muscle. This muscle was chosen as the stimulation site due to its accessibility in creating DOMS and performing pressure stimulations. Temporal summation (TS) and conditioned pain modulation (CPM) were used to describe central acting pain mechanisms.

5.1 Experiment design

The experiment design should encompass the following steps:

- Normalization of pressure stimulations
- TS and CPM as control paradigms
- A pain model introducing DOMS
- OA using noxious pressure stimulations
- OA using noxious heat stimulations

A pilot study was performed in order to specify how to design and perform OA using pressure stimulations (Appendix A on page 45).

Cuff

The main tool for assessing pressure pain in this experiment and to fulfill the performance specification described in Section 5.1 was computerized cuff algometry (CPA). Compared to e.g. hand-held algometry the intertester bias is less influent due to the absence of manual involvement when applying the pressures [Polianskis et al., 2001]. CPA is a recognized method for assessing deep tissue pain [Polianskis et al., 2002, Skou et al., 2012; 2013b].

The pressure stimulations were mainly performed with a double chambered 13 cm wide pneumatic tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) placed around the shin of the dominant leg at the level of the belly of the gastrocnemius-soleus muscles in accordance with [Polianskis et al., 2001]. It is a high-pressure silicone cuff in which the equally sized chambers are separated lengthwise. Furthermore a second cuff, a single chambered 7.5 cm wide cuff (VBM Medizintechnik GmbH, Sulz, Germany) with equal characteristics, was applied to assess CPM. A computer-controlled air-compressor (NociTech Aps, Denmark) was used to regulate the compression rate continuously. The compressor was controlled by a pressure-control program written in LabView5 (National Instruments, Austin, Texas, U.S.). Safety precautions were made: The maximal pressure limit was set at 100 kPa, and the inflation could be terminated both by a hand-held release button and from the computer program.

Further procedures involving cuff algometry are described in detail in the following sections.

PPT-PPTol

It is known that thermal stimuli of $43-51\,^{\circ}\mathrm{C}$ are perceived as painful [Price et al., 1984]. Thus, when performing experiments with heat stimuli the same stimulation can be used for all subjects. However the pilot study showed that different pressure intensities applied by a double chambered cuff resulted in various pain ratings among subjects. To ensure that each subject experienced a painful stimulus, which was not above their tolerance level, the pressure pain threshold (PPT) and the pressure pain tolerance threshold (PPTol) were measured, and the applied pressures were thereby normalized to the individual subject's pain levels.

PPT and PPTol were measured for each subject. The double chambered cuff was gradually inflated at a rate of 1 kPa/s. To determine PPTs subjects were instructed to indicate on an electronic VAS (eVAS) when the sensation of pressure first became painful. To examine PPTol, subjects were instructed to push a response button on the eVAS when the pressure sensation reached their tolerance level.

Offset analgesia

The main aim of this experiment was to investigate the existence of OA in deep muscles pain induced by noxious pressures. The method of Grill and Coghill [2002] was adapted and modified in order to create mechanical OA trials consisting of three contiguous phases: an initial pressure (P1), an incremental increase to a second pressure (P2) followed by a decrease to a third pressure (P3), which is equivalent to P1 (Figure 5.1). Furthermore mechanical constant trials were conducted in order to calculate the magnitude of a potential OA effect and to evaluate whether the potential decrease in pain ratings were caused by adaptation of primary afferents.

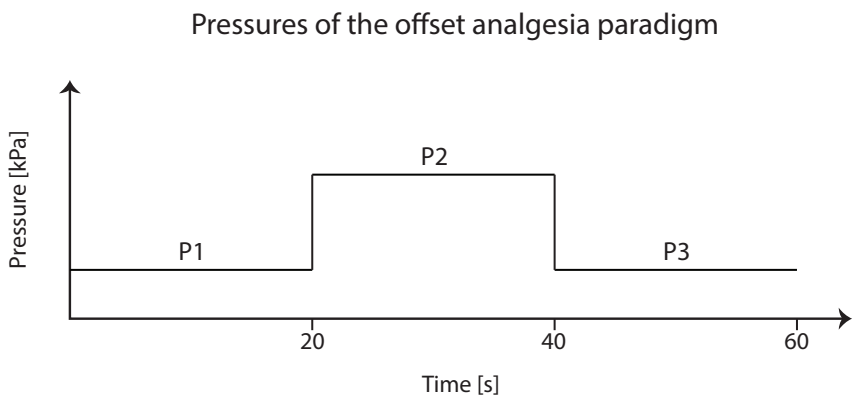

Figure 5.1: *Illustration of the pressures in the OA paradigm.*

The experiment was performed in four condition with various pressures as seen in Table 5.1. The four conditions and the pressure stimulations within each condition were randomized (see an example in Table vreftab:offsetparadigm.

Randomization of pressure stimuli			
Condition 4	Offset (B1,B,B1)	Constant high (B)	Constant low (B1)
Condition 3	Constant low (B2)	Offset (B2,B,B2)	Constant high (B)
Condition 1	Constant high (A)	Constant low (A1)	Offset (A1,A,A1)
Condition 2	Constant high (A)	Offset (A1,A,A1)	Constant low (A2)

Table 5.1: *An example showing how the four stimulation conditions could be presented to a subject*

Each condition holds a mechanical OA trial and two mechanical constant trials:

- A constant high pressure (A or B, 60 s duration)
- A constant low pressure (A1, A2, B1 or B2, 60 s duration)
- An offset pressure (P1: A1, A2, B1 or B2, 20 s duration), an incremental increase to a second offset pressure (P2: A or B, 20 s duration) and an incremental decrease to a third offset pressure (P3: A1, A2, B1 or B2, 20 s duration)

The pressures were determined from the PPT-PPTol interval (Figure 5.2). PPT corresponded to 0 % of the interval and PPTol was equal to 100 %. The two high pressures were defined as A = 50 % and B = 75 %. The four low pressures A1, A2, B1 and B2 were defined as 30 %, 40 %, 55 % and 65 % respectively, see Figure 5.2.

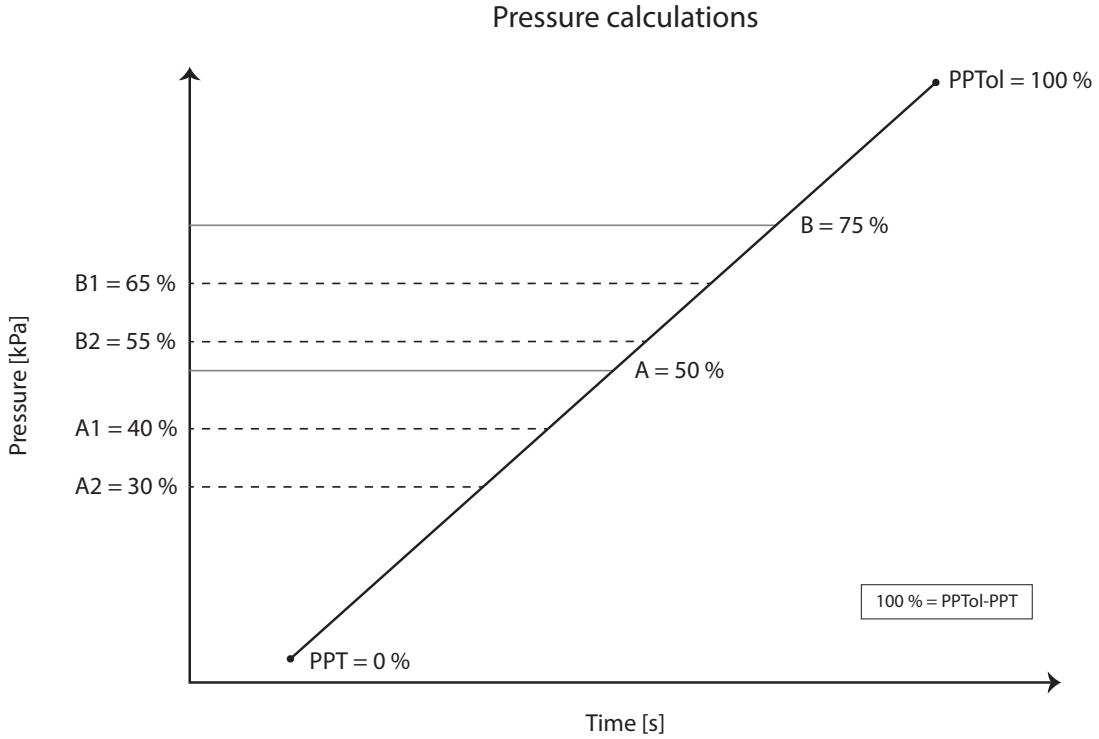

Figure 5.2: *Definitions of the different pressures applied during the mechanical stimulations.*

Furthermore a control experiment applying noxious heat stimuli was conducted to clarify that the subjects did elicit OA as described by Grill and Coghill [2002]. A 30x30 mm thermode with rise and fall rate of 6 °C/s (Medoc, Ramat Yishai, Israel) was used to deliver thermal stimuli to the ventral surface of the forearm. The thermode was fixed to the dominant forearm 5 cm distal to the elbow joint with a velcro strap and the temperature was maintained at a baseline of 35 °C. The thermode was connected to a Medoc Pathway pain and sensory evaluation system (Medoc, Ramat Yishai, Israel) from which the thermal stimulations was controlled.

According to the study conducted by Grill and Coghill [2002] the heat OA trials involved three contiguous phases: an initial temperature (T1, 48 °C), an incremental increase to a second temperature (T2, 49 °C) followed by a decrease to a third temperature (T3, 48 °C), which is equivalent to T1. Furthermore heat constant trials of 48 °C) were conducted in order to calculate the magnitude of a potential OA effect and to evaluate whether the potential decrease in pain ratings were caused by adaptation of primary afferents. The stimulus trial consist of two thermal stimuli:

- A constant temperature (T1, 30 s duration)
- An offset temperature (T1, 5 s duration), an incremental increase to a second offset temperature (T2, 5 s duration) and an incremental decrease to the third offset temperature (T3, 20 s duration)

Temporal summation

Measurements of TS were included as control paradigms. TS results in temporal alterations during noxious stimuli. Thus, measurements of TS may provide additional information about temporal processes of the nociceptive system. [Martucci et al., 2012a] Stability changes have been observed between OA and TS. Both phenomena reflect pain modulating mechanisms, however they are not necessarily mediated by the same mechanisms as the effect of TS is altered by opioids and OA is opioid independent [Martucci et al., 2012a, Price et al., 1985, Smith, 2009].

The TS experiment was conducted in two series with the pressures A and B (defined in Figure 5.2 on the preceding page). The TS paradigms consisted of sequences of ten stimuli (1 s duration and 1 s interstimulus interval) in accordance with Graven-Nielsen and Arendt-Nielsen [2010] (Figure 5.3). In order to avoid movement of the cuff during inflations a constant non-painful baseline pressure of 5 kPa was kept between the individual pressures in accordance with Skou et al. [2013a].

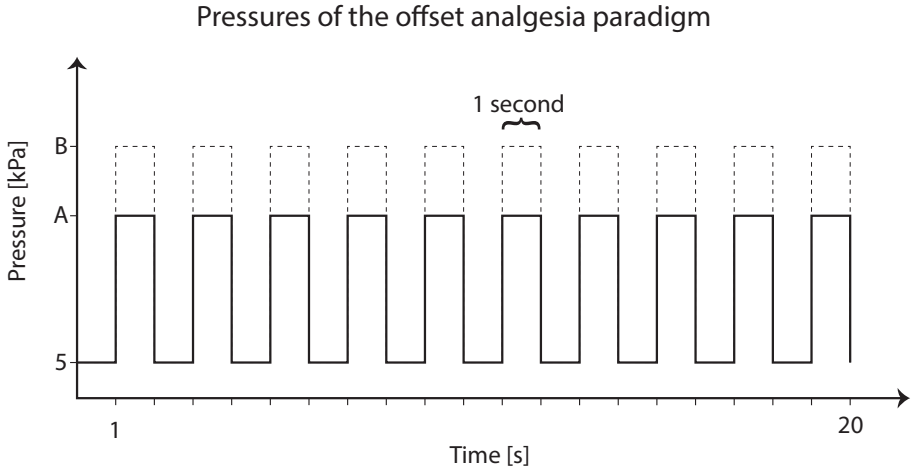

Figure 5.3: *Illustration of the two temporal summation paradigms with pressure A and B with ten stimulations.*

Computationed pain modulation

CPM is a pain modulating mechanism as TS and OA. Contrary to TS, CPM is a spatial pain inhibitory mechanism where a heterotopic conditioned stimulus decreases the pain perception caused by another noxious stimulus[Arendt-Nielsen and Yarnitsky, 2009]. Changes between TS, CPM and OA have been observed during opioid treatment. Opioids causes a decrease in pain facilitation in TS and decrease in pain inhibition in CPM. Both TS and CPM are therefore opioid dependent mechanism while OA is independent [Martucci et al., 2012a]. Measurements

of CPM was included as a control paradigm in the study to investigate spatial pain modulating mechanisms.

The CPM procedure was separated in two parts with the first part serving as a calculation of the heterotopic conditioned pressure. The single chambered cuff was placed around the belly of the biceps brachii muscle off the contralateral arm, and a continuously inflating pressure at a rate of 1 kPa/s was applied. The subject was instructed to continuously rate the pain on an electronic VAS from the first sensation of pain and to push a response button when PPTol was reached. According to a study conducted by Graven-Nielsen and Arendt-Nielsen [2010] the heterotopic conditioned pressure used to evoke CPM had to be painful. Thus, the pressure during the CPM procedure was determined to be the pressure corresponding to a pain rating of 5.0 on the VAS. In part two a constant pressure corresponding to a VAS 5 was applied to the single chambered cuff placed around the contralateral arm. Concurrent a continuously increasing pressure at a rate of 1 kPa/s was applied to the double cambered cuff placed on the dominant leg and a PPT measure was re-assessed (see Figure 5.4). The subject was instructed to ignore the noxious pressure on the arm and continuously rate the pain on the eVAS and to push a response button when PPTol was reached.

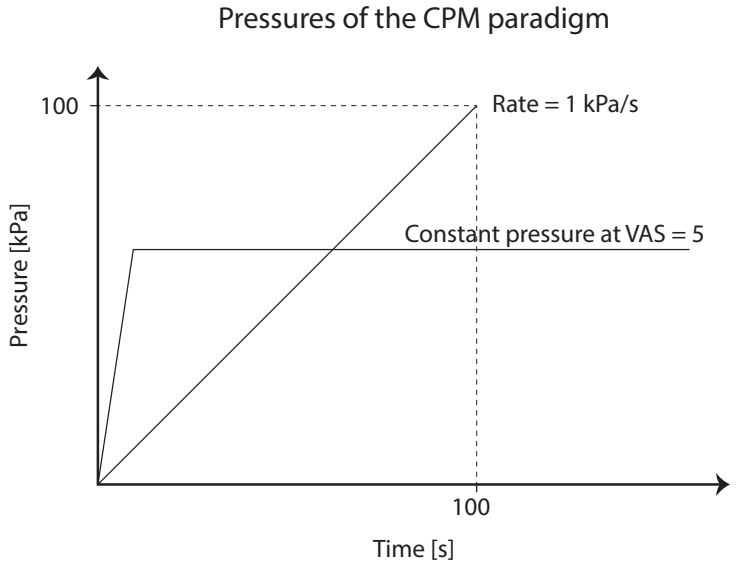

Figure 5.4: *Illustration of the CPM paradigm with a PPT measurement and a concurrent heterotopic conditioned pressure corresponding to a VAS 5 rating.*

Delayed onset muscle soreness

A DOMS model was applied in this study to evaluate the effect of pain/muscle soreness on OA induced by different noxious pressure stimuli. Thereby evaluating the effect of sensitized nociceptors in the affected muscle on OA.

The model used is a modification of the model described by Kanda et al. [2013]. The subjects were instructed to perform body weighted calf-raise exercises, which included repetitive eccentric muscle contractions, with their dominant leg. They performed 10 sets of the exercise (8 sets of 20 repetitions and 2 sets 40 repetitions) at 0.5 Hz. In between each set a rest period of 3 min was applied. On day 3 an eVAS was used to rate DOMS with the extremes 'no pain' and 'worst imaginable pain'.

5.2 Experiment procedure

17 healthy subjects (12 males and 5 females), age 24.8 ± 1.4 years (mean ± SD), participated in this study. The aim of the experiment was to investigate the existence of OA in deep muscle pain. Thus, subjects had to meet certain inclusion and exclusion criteria listed below:

Inclusion criteria

- Healthy men and women
- 18-60 years

Exclusion criteria

- Participating women must not be pregnant
- Drug addiction, defined as the use of cannabis, opioids or other drugs
- Current use of medications that may affect the experiment (pain relievers, psychotropic drugs, etc.)
- Previous or current neurological diseases
- Lack of interpersonal skills
- Consumption of alcohol, caffeine, nicotine or painkillers 24 hours before the study day
- Acute or chronic pain
- Tattoos or moles in the stimulation area
- Participation in other experiments one week before the study day and in parallel with the study

After an explanation of the experiment, all subjects provided written informed consent, acknowledged that they understood the experiment, and that they were free to terminate stimuli or withdraw from the study at any time. The experiment was carried out according to the experimental protocol (see Appendix B on page 49).

The experiment was divided into three days:

Day 1

1. Control experiment with OA heat stimulation

Day 2

1. Training
2. PPT and PPTol measurements
3. OA pressure stimulation
4. TS
5. CPM
6. DOMS

Day 3

1. PPT and PPTol measurements
2. OA pressure stimulation
3. TS
4. CPM

During the measurements of OA and TS, the applied noxious pressures were randomized in order to avoid hidden correlations within the experiment. Furthermore rest period of 60 s were incorporated in between each stimulus.

6

Data analysis

The data was analyzed and various parameters were calculated. Relevant calculations and methods are clarified in the following sections and lastly important statistical methods are described.

6.1 Validation of pain model

I order to apply the pain model created from DOMS a validation was performed. The validation investigated whether DOMS was present or not by examining data from PPT measurements and the subject's pain rating prior to the experiments on day three. Pain ratings above 3.0 on the eVAS was considered a pain response due to DOMS. Furthermore a decrease in PPT in the experiment on day three was considered a pain response of DOMS.

The PPTs were calculated by detection of the first reactions on the eVAS. These were detected by an algorithm in MATLAB. In Figure 6.1 an example of PPT detection is illustrated. A deflection on the VAS was detected and a corresponding pressure was read off the left y-axis.

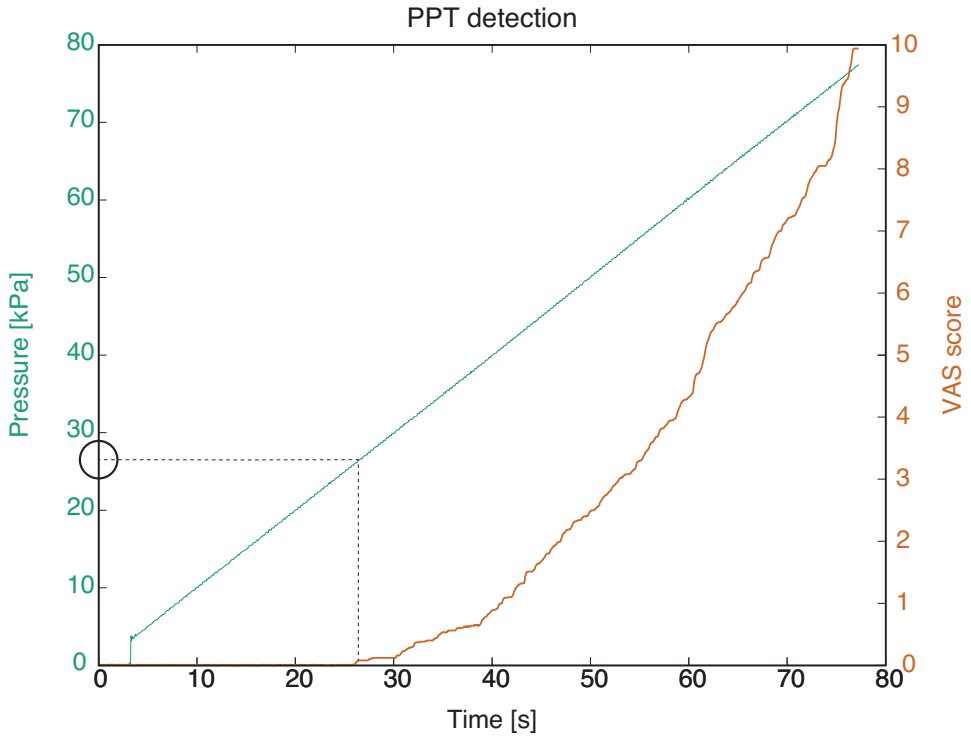

Figure 6.1: *Example of a PPT detection. A reaction on the eVAS was detected and a reading of the corresponding pressure was made on the left y-axis.*

6.2 Offset analgesia

From the continuous VAS ratings of mechanical and heat stimulations different VAS scores were determined. If a significant OA effects was observed the VAS ratings were used to calculate the magnitude of OA for noxious pressure and noxious heat stimuli respectively.

Mechanical stimulations

Two parameters were extracted from the continuous data:

- *MinOffset*
- *MinConstant*

MinOffset was defined as the minimum VAS score during the P3 pressure (40-60 s) illustrated in gray in Figure 6.2. *MinConstant* was defined as the average VAS score for the constant trial within the same time interval as *MinOffset* was detected (interval illustrated with dashed lines in Figure 6.2). In case *MinOffset* did not correspond to a time interval but a single time point *MinConstant* was defined as the VAS score at that single time point.

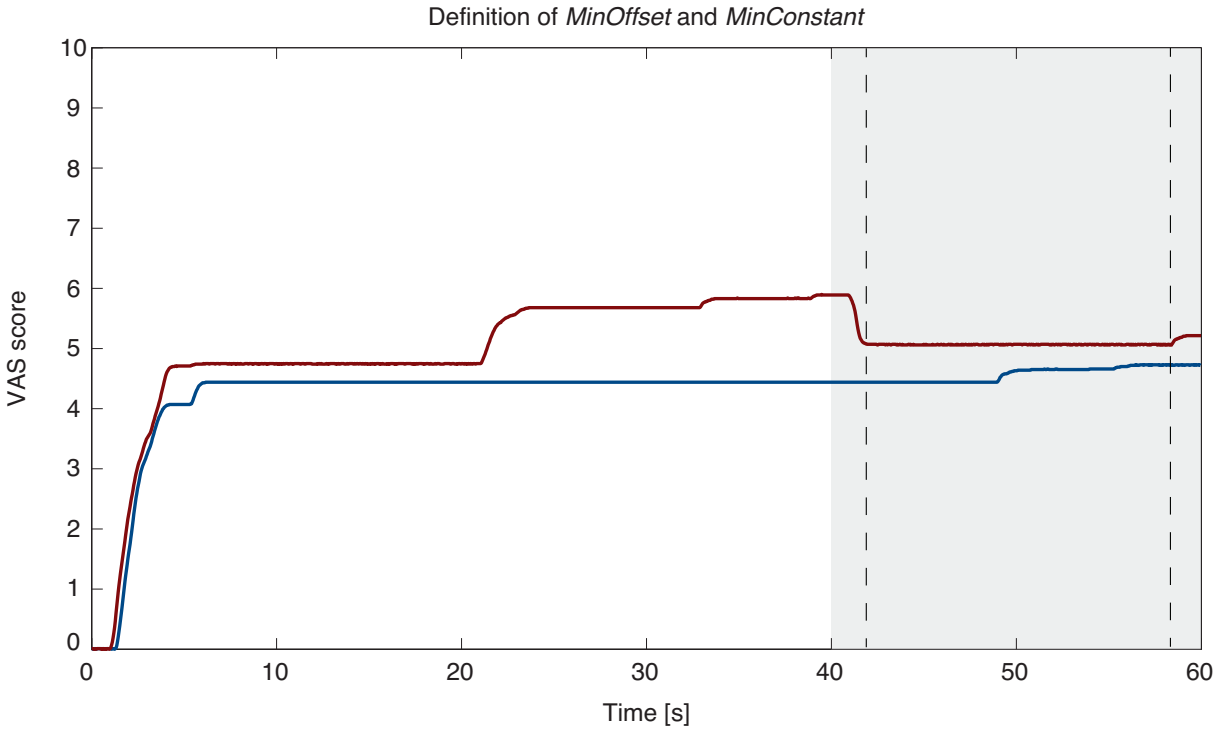

Figure 6.2: *MinOffset was the minimum VAS score during the P3 pressure stimulus (gray area). MinConstant was the average VAS score for the constant trial within the same time interval as MinOffset was detected (marked with dashed lines).*

The potential effect of OA (*MagnitudeOA*) was calculated by subtracting the VAS scores of *MinConstant* from VAS scores of *MinOffset*.

Heat stimulations

MinOffset, *MinConstant* and *MagnitudeOA* during heat stimulations were calculated in the same way as in the mechanical stimulations (described in the previous section), here applying VAS scores for heat stimulations instead. Figure 6.3 on the facing page illustrates the average VAS scores of heat stimulations where *MinOffset* is represented at a single time point (marked with a dashed line). *MinConstant* was therefore defined as the VAS score at that same time point.

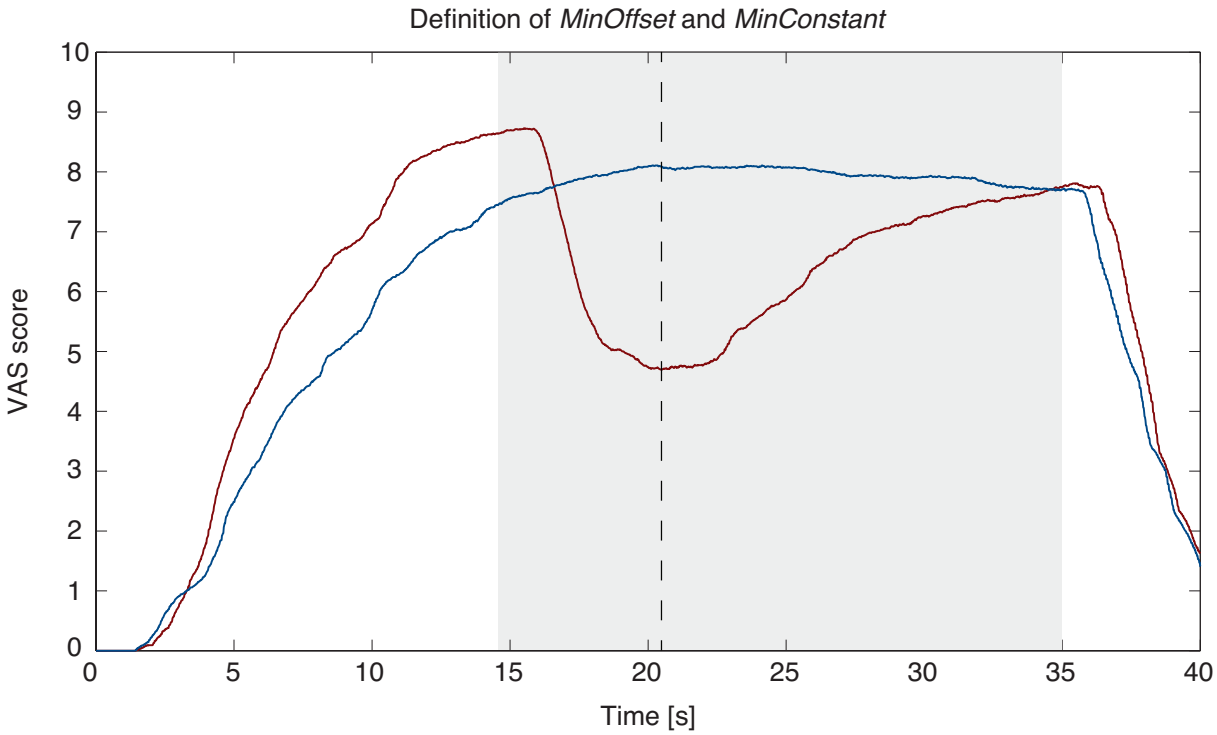

Figure 6.3: *MinOffset was the minimum VAS score during the T3 temperature stimulus (gray area). MinConstant was the average VAS score for the constant trial within the same time interval as MinOffset was detected. In the heat stimulations this interval was represented as a single time point (marked with a dashed line) hence MinConstant was defined as the VAS score at that single time point.*

6.3 Temporal summation

TS was calculated from the data using mechanical stimulations. The data was analyzed in order to calculate alterations in the pain ratings. The magnitude of TS has previously been calculated as the difference in pain rating between initial and final stimulus [Yarnitsky and Pud, 2004].

Due to the reaction time of the subjects the first pain ratings was defined as the VAS score at time equal two seconds (iVAS in Figure 6.4 on the next page). This figure shows the average pain rating of all subjects. In order to detect the largest magnitude of TS the final pain rating (fVAS) was defined as the maximum rating on the VAS (fVAS in Figure 6.4 on the following page). The magnitude of TS was then calculated as the increase in VAS score from iVAS to fVAS.

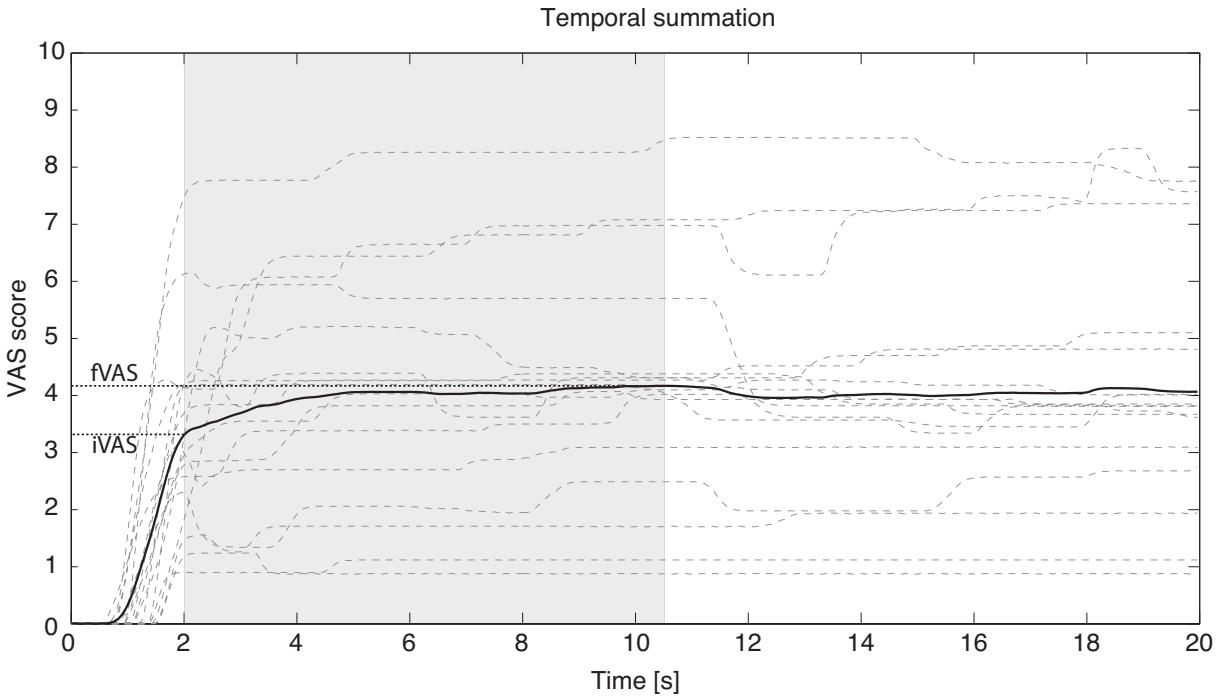

Figure 6.4: *Calculations of the magnitude of temporal summation from the averaged pain ratings.*

6.4 Conditioned pain modulation

In order to verify that the subjects were able to elicit the central pain mechanism CPM, it was investigated how CPM affected the pressure at VAS 7 in PPT measurements of the dominant leg. Under normal conditions when the subjects do not experience any pain an increase in pressure at VAS 7 was expected during CPM. Whereas on day three of the experiment where subjects have DOMS no change in pressure was expected due to already sensitized nociceptors [Lewis et al., 2012].

In Figure 6.5 on the next page an example of CPM's effect on PPT and the VAS 7 detection is illustrated. A change in pressure was determined from the right y-axis.

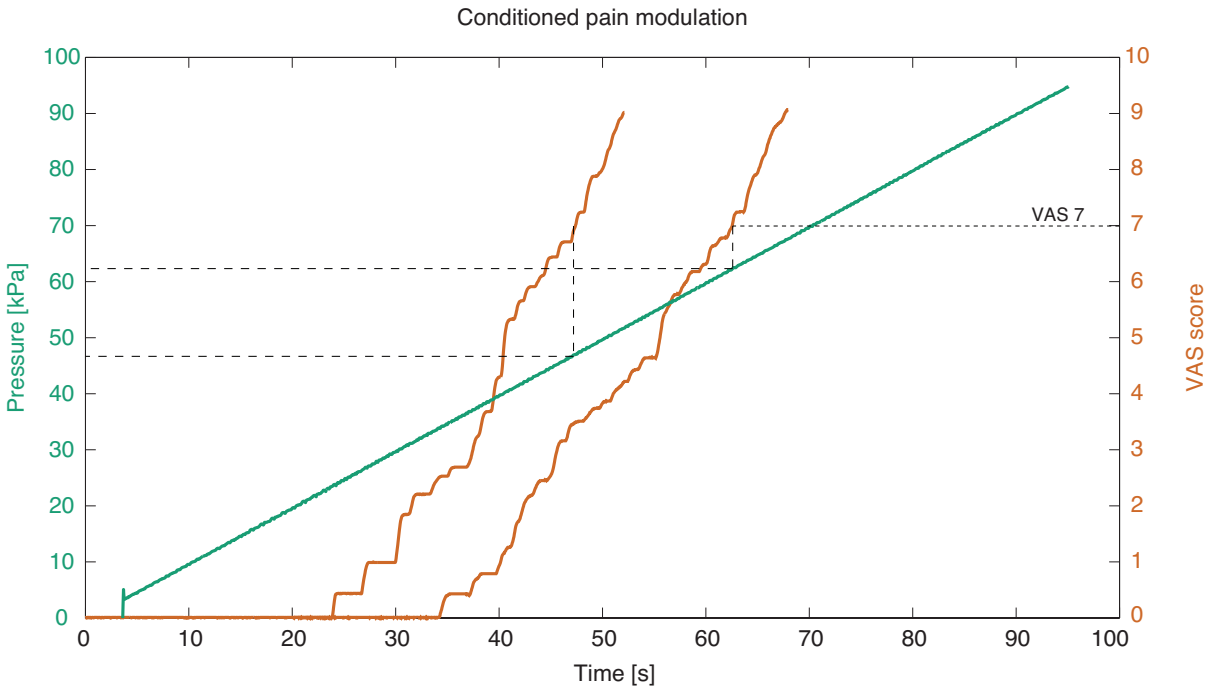

Figure 6.5: *Example of a pressure detection at VAS 7 when a conditioned pain stimuli was applied to the contralateral arm. The VAS 7 score was detected and a reading of the corresponding pressure was made on the left y-axis.*

6.5 Statistics

The statistical analysis conducted in this study is described in the following sections. To get an overview and ease the description, the flow chart in Figure 6.6 elaborates when the different methods were applied.

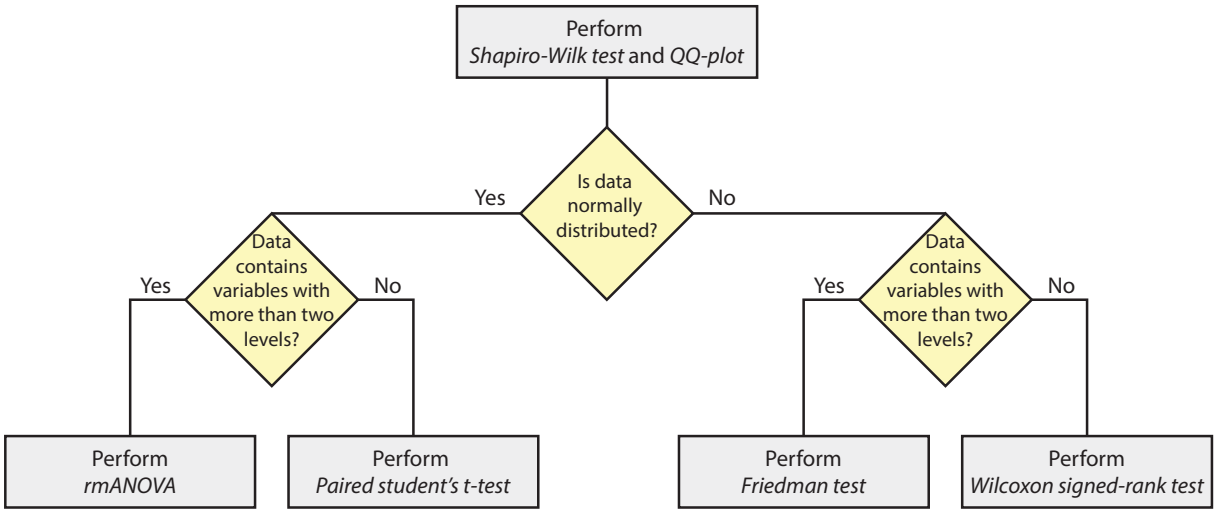

Figure 6.6: *Flow chart illustrating the statistical analysis.*

Shapiro-Wilk test

Many statistical procedures assume that data is normally distributed, and a violation of this assumption can result in invalid or unreliable interpretations of the data. Thus, it is important to investigate whether this assumption is valid before proceeding with the statistical analysis. [Razali and Wah, 2011]

According to Razali and Wah [2011] the Shapiro-Wilk test is the most powerful normality test for small sample sizes, but it should be combined with a graphical technique as verification of the result [Razali and Wah, 2011]. It was therefore decided to apply the Shapiro-Wilk test in combination with the graphical technique quantile-quantile plot (QQ-plot) to determine whether the data from the parameters of interest were normally distributed.

The following test hypotheses were stated with an α value of 0.05:

- H_0 = data is normally distributed
- H_A = data is not normally distributed

The null hypothesis is rejected if the p-value is less than 0.05 (the alpha value).

A QQ-plot displays the sample quantiles of the data against theoretical quantiles from a normal distribution. Thus, if the data is normally distributed, the plot will closely represent a linear line [MathWorks, 2014a].

Paired student's t-test

Paired student's t-tests were applied on normally distributed data with variables containing no more than two levels. The test was therefore performed on the parameters PPT and PPT(DOMS) to test if any statistically significant difference was found between baseline measures of PPT compared with PPT measures during the pain model of DOMS. Furthermore paired student's t-tests were performed to test if there was a significant difference between PPT pressure at VAS 7 and PPT pressure at VAS 7 during CPM and if this was effected by DOMS. The following test hypotheses were stated with an α value of 0.05:

- H_0 = there is no statistical significant difference
- H_A = there is a statistical significant difference

P-values below the α value of 0.05 would result in a rejection of the null hypothesis [Zar, 2010].

Wilcoxon signed-rank test

Wilcoxon signed-rank test is an alternative to the paired-student's t-test, and it was applied on non-normally distributed data with variables containing no more than two levels [Oyeka and Ebuh, 2012, Zar, 2010]. The Wilcoxon signed-rank test was performed on the parameters *MinConstant(Heat)* and *MinOffset(Heat)* to test if any statistically significant difference could be found. The following test hypotheses were stated with an α value of 0.05.

- H_0 = there is no statistical significant difference
- H_A = there is a statistical significant difference

If the obtained P-value was less than the alpha value the null hypothesis was rejected, at a 5 % significance level [MathWorks, 2014b].

Repeated measures ANOVA and Bonferroni

Repeated measures ANOVAs (rmANOVAs) with the factors Pressure, Stimulus and DOMS were used to test if there were any significant difference between *MinOffset* and *MinConstant* at the four conditions described in Section 5.1 on page 14. Furthermore if a significant difference was observed between baseline measurements and DOMS measurements at the four pressures. Additionally an rmANOVA containing similar factors was applied in TS to test for significant differences between VAS scores corresponding to initial and final pressures at the two pressures A and B and between baseline measurements and DOMS measurements.

In case a statistical significant difference was found in the rmANOVA a Bonferroni post hoc test was applied. The Bonferroni test clarifies between which factors the statistical difference was found and deals with multiplicity within the ANOVA. [Streiner and Norman, 2011]

7

Results

The experimental trials were divided into two groups: healthy subjects and subjects having DOMS. When the subjects are experiencing DOMS they should resemble a patient group suffering from a pain condition. Thus, the subjects are characterized as healthy in day one and two.

7.1 Normal distribution

The Shapiro-Wilk's test was performed to test whether the parameters of interest were normally distributed. The P-values from this test are summarized in the following two tables. Asterisks indicate that the parameters are not normally distributed. Table 7.1 holds the P-values of mechanical OA trials and mechanical constant trials, with all parameters of interest showing normal distributions, expect for *MinOffset(DOMS)* in Condition 1.

P-values from Shapiro-Wilk test				
	Condition 1	Condition 2	Condition 3	Condition 4
MinOffset	0.194	0.260	0.664	0.669
MinContant	.0197	0.776	0.653	0.650
MinOffset(DOMS)	0.039*	0.410	0.587	0.064
MinContant(DOMS)	0.250	0.324	0.683	0.786

Table 7.1: *P-values from the Shapiro-Wilk test, stating that all parameters are normally distributed except from MinContant(DOMS).*

Table 7.2 on the next page contains P-values from the Shapiro-Wilk test regarding PPT, temporal summation (TS) at the two pressures A and B, conditioned pain modulation (CPM) and the control experiment with heat stimulations. *MinConstant(heat)* is the only parameter of interest which is not normally distributed in this table.

P-values from Shapiro-Wilk test	
PPT	0.282
PPT (DOMS)	0.227
TS1 (initial)	0.196
TS1 (final)	0.084
TS1 (initial,DOMS)	0.821
TS1 (final,DOMS)	0.748
TS2 (initial)	0.685
TS2 (final)	0.068
TS2 (initial,DOMS)	0.127
TS2 (final,DOMS)	0.263
CPM	0.140
CPM (DOMS)	0.176
MinOffset(heat)	0.948
MinConstant(heat)	0.014*

Table 7.2: *P-values from the Shapiro-Wilk test, stating that all parameters of interest are normally distributed except from MinConstant(heat).*

QQ-plots were made to verify the results of the Shapiro-Wilk test. Figure 7.1 illustrates two examples; one parameter which is not normally distributed and one that is, respectively. By comparing the results in Table 7.2 with the QQ-plots in Figure 7.1 it can be seen that the results are consistent. *MinConstant(heat)* is not normally distributed whereas *MinOffset(heat)* is normally distributed

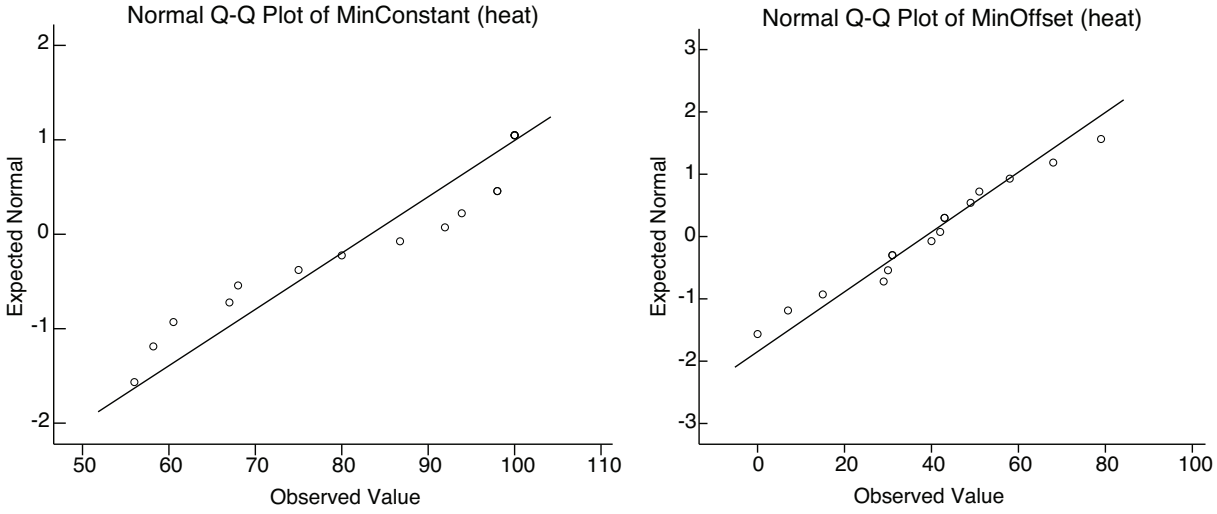

Figure 7.1: *The QQ-plots show that MinConstant(heat) is not normally distributed whereas MinOffset(heat) is normally distributed.*

7.2 Validation of pain model

The applied pain model DOMS was validated by measuring PPTs and by recording the subject's pain rating at arrival on day tree. From these measurements a mean and standard deviation was calculated as seen in Table 7.3 on the next page. The mean PPT is 22.48 kPa with a standard deviation of 12.3 kPa. During DOMS the mean is reduced to 17.36 with a standard deviation of 5.91. The subjects mean pain rating on day three was 3.71 ± 2.49.

Mean values and SD	
PPT	22.48 ± 12.30
PPT(DOMS)	17.36 ± 5.91

Table 7.3: *Mean and standard deviation of PPTs.*

A paired student's t-test was applied in order to test if there is a significant difference in mean values between PPT and PPT(DOMS). This is illustrated in Figure 7.2 with the mean values and the corresponding standard deviations.

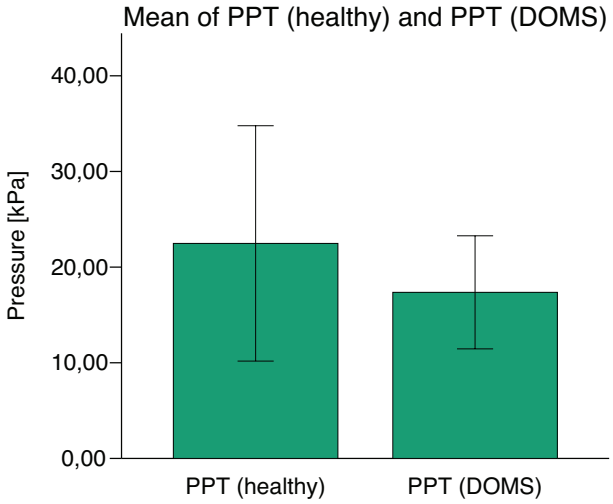

Figure 7.2: *Mean values and standard deviation of PPT measurements.*

The results from the paired student's t-test show that there is a significant difference in mean between PPT and PPT(DOMS). The P-value is 0.029 and thus less than the significance level 0.05.

P-value from the paired student's t-test	
PPT-PPT(DOMS)	0.029*

Table 7.4: *The P-value is less than 0.05 indicating a significent difference in mean.*

7.3 Offset analgesia

This section contains the key results obtained from the data analysis in Section 6.2 on page 20 regarding the evaluation of offset analgesia (OA) during noxious heat and noxious pressure stimulation. A further discussion of the results and figures in this section can be found in Chapter 8

Heat stimulation

The results from the control experiment with noxious heat stimuli are presented in the following. Figure 7.3 on the following page illustrates the average VAS scores in the heat OA trials and heat constant trials. The solid red curve represents the mean of the continuous VAS scores corresponding to the temperature stimuli, which follows a normal OA paradigm (48-49-48 °C). The solid blue curve is the mean of the continuous VAS scores corresponding to a temperature stimuli of 48 °C. Standard deviations are illustrated with dashed lines.

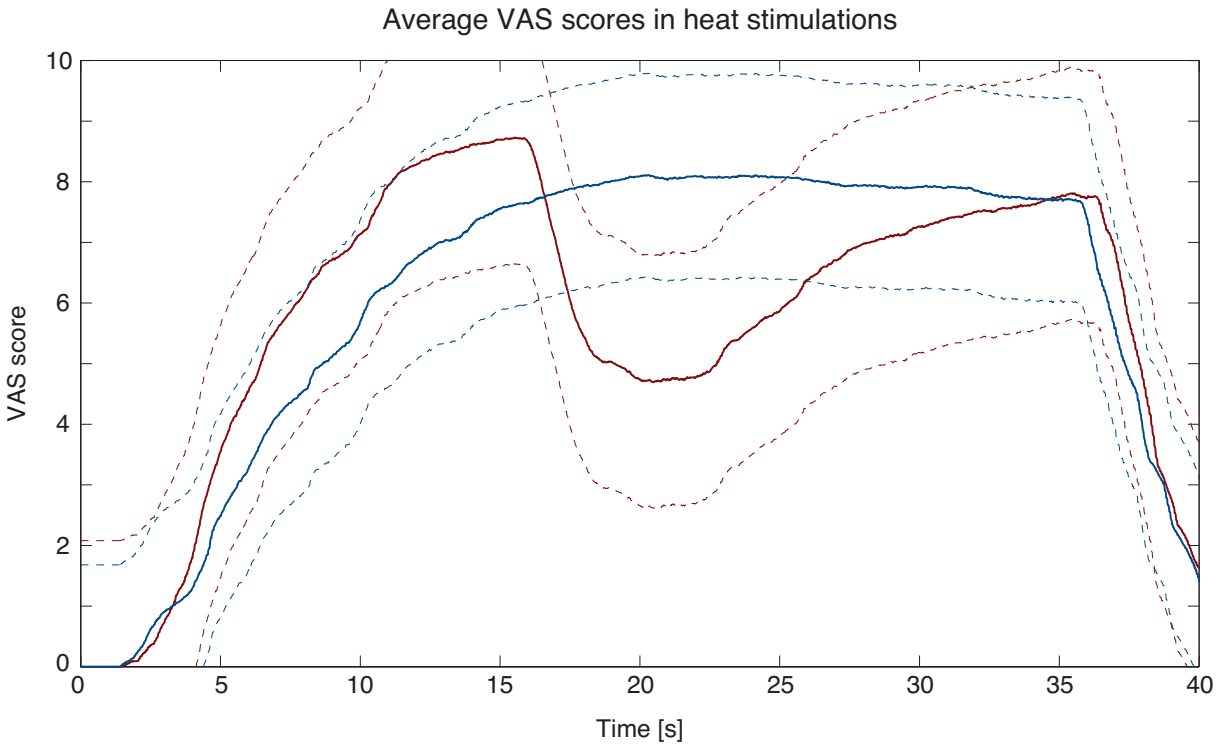

Figure 7.3: *The solid red curve represents the average of the continuous VAS scores corresponding to the temperature stimuli which follows a normal OA paradigm (48-49-48 °C). The solid blue curve represents the mean VAS scores corresponding to a constant temperature stimulus at 48 °C. Standard deviations are illustrated with dashed lines.*

From the recorded data the parameters *MinOffset(heat)* and *MinConstant(heat)* was calculated. The mean and standard deviations of these parameters are listed in Table 7.5.

Mean values and SD	
MinOffset	3.85 ± 2.08
MinConstant	8.33 ± 1.68

Table 7.5: *Mean and standard deviations of the parameters MinOffset(heat) and MinConstant(heat).*

The mean values and corresponding standard deviations of *MinOffset(heat)* and *MinConstant(heat)* during noxious heat stimuli are illustrated as bar plots in Figure 7.4. The red bar illustrates the mean of *MinOffset(heat)* and the blue bar represents the mean of *MinConstant(heat)*.

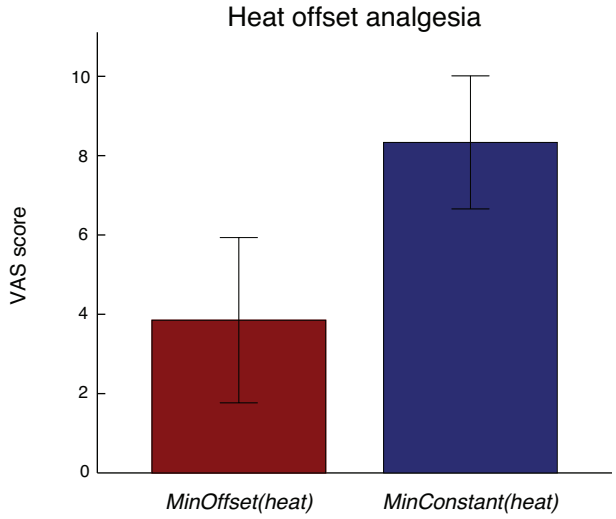

Figure 7.4: *The mean and standard deviations of MinOffset(heat) and MinConstant(heat).*

The Wilcoxon signed-rank test was used to test the hypothesis that there is a statistical significant difference between *MinOffset(heat)* and *MinConstant(heat)*. If *MinOffset(heat)* is significant smaller than *MinConstant(heat)* this would indicate that OA exists during noxious heat stimulations. The result shows that there is a statistical significant difference between the mean of *MinOffset(heat)* and *MinConstant(heat)* ($P = 0.00044$), indicating that OA exists during noxious heat stimulation in healthy subjects.

Pressure stimulation

The results from healthy subjects and subjects having DOMS is presented in the following sections.

Healthy

Figure 7.5 illustrates the mean VAS scores of the mechanical OA trials and the mechanical constant trials in four conditions. The red curves represent the mean of the continuous VAS scores during the mechanical OA trials and the blue curves are the mean of the continuous VAS scores during the mechanical constant trials. Standard deviations are illustrated with dashed lines.

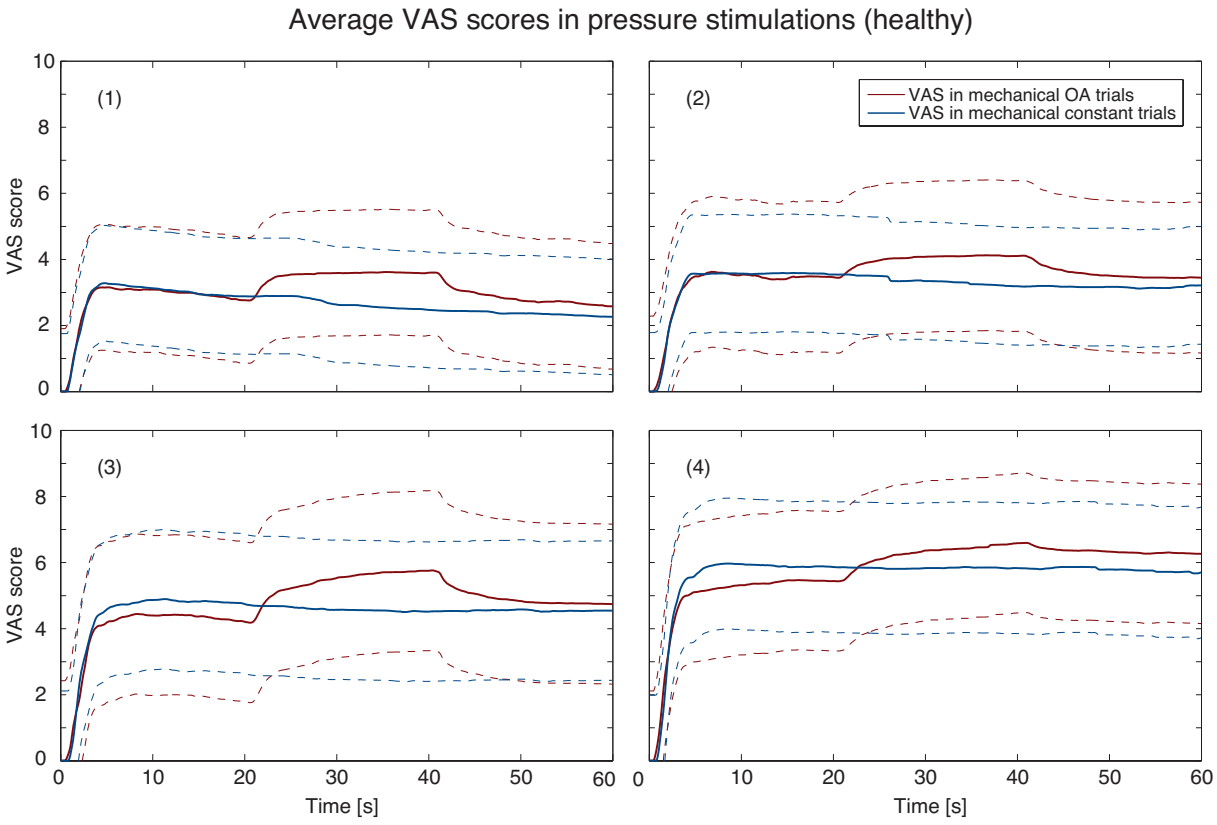

Figure 7.5: *The red curves represent the mean of the continuous VAS scores during mechanical OA trials and the blue curves represent the mean of the continuous VAS scores during mechanical constant trials. Standard deviations are illustrated with dashed lines.*

The parameters *MinOffset* and *MinConstant* were calculated from the recorded data. The mean and standard deviations of these parameters are listed in Table 7.6 on the following page.

Mean values and SD				
	Condition 1	Condition 2	Condition 3	Condition 4
MinOffset	2.45 ± 1.9	3.36 ± 2.28	4.7 ± 2.42	6.14 ± 2.11
MinContant	2.38 ± 1.75	3.15 ± 1.78	4.52 ± 2.11	5.78 ± 1.98

Table 7.6: *The mean and standard deviations of MinOffset and MinConstant in the four conditions.*

The mean values and corresponding standard deviations of *MinOffset* and *MinConstant* at the four conditions are illustrated in Figure 7.6. The red bars represent the mean of *MinOffset* and the blue bars show the mean of *MinConstant*.

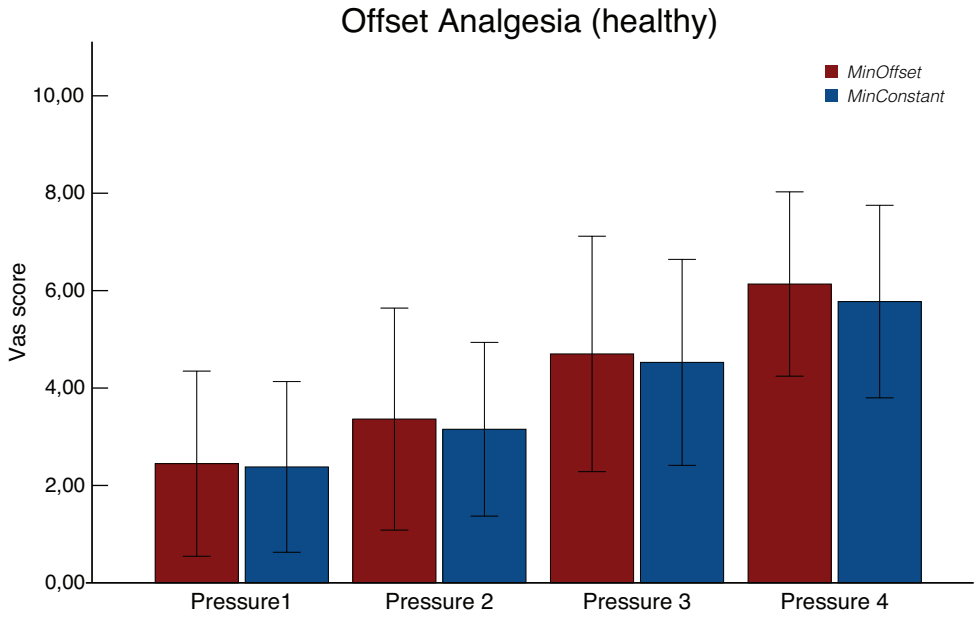

Figure 7.6: *Mean and standard deviations of the parameters MinOffset and MinConstant.*

A rmANOVA was used to test the hypothesis that there is a statistical significant difference between *MinOffset* and *MinConstant*, hence if *MinOffset* is significant smaller this would indicate that OA exists during noxious pressure stimuli. The rmANOVA shows that there is no statistical significant difference between the mean of *MinOffset* and *MinConstant* ($F(3) = 0.227$, $P = 0.750$), indicating that OA does not exist during noxious pressure stimulation in healthy subjects.

DOMS

Figure 7.7 on the facing page illustrates the mean VAS scores of the subjects having DOMS in the mechanical OA trials and the mechanical constant trials in four conditions. The red curves represent the mean of the continuous VAS scores during the mechanical OA trials and the blue curves are the mean of the continuous VAS scores during the mechanical constant trials. Standard deviations are illustrated with dashed lines.

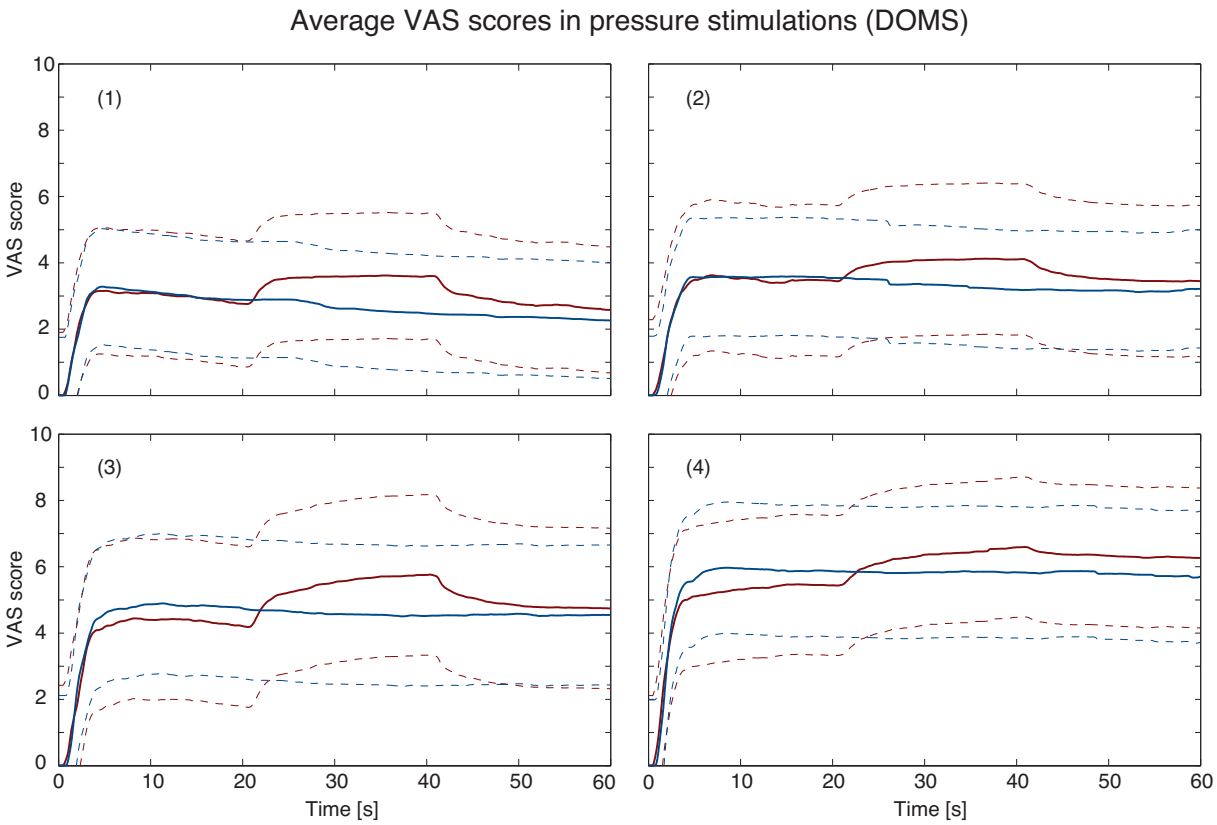

Figure 7.7: *The red curves represent the mean of the continuous VAS scores during the mechanical OA trials and the blue curves represent the mean of the continuous VAS score during the mechanical constant trials. Standard deviations are illustrated with dashed lines.*

From the recorded data the parameters *MinOffset(DOMS)* and *MinConstant(DOMS)* was calculated. The mean and standard deviations of these parameters are summarized in Table 7.7.

Mean values and SD				
	Condition 1	Condition 2	Condition 3	Condition 4
MinOffset(DOMS)	2.69 ± 2.36	3.6 ± 2.62	4.77 ± 2.14	6.25 ± 2.19
MinContant(DOMS)	2.53 ± 1.78	3.33 ± 2.34	5.17 ± 1.61	5.43 ± 2.1

Table 7.7: *The mean and standard deviations of MinOffset(DOMS) and MinConstant(DOMS) in the four conditions.*

The mean values and corresponding standard deviations of *MinOffset(DOMS)* and *MinConstant(DOMS)* of the four conditions are illustrated in Figure 7.8 on the following page. The red bars represent the mean of *MinOffset(DOMS)* and the blue bars represent the mean of *MinConstant(DOMS)*.

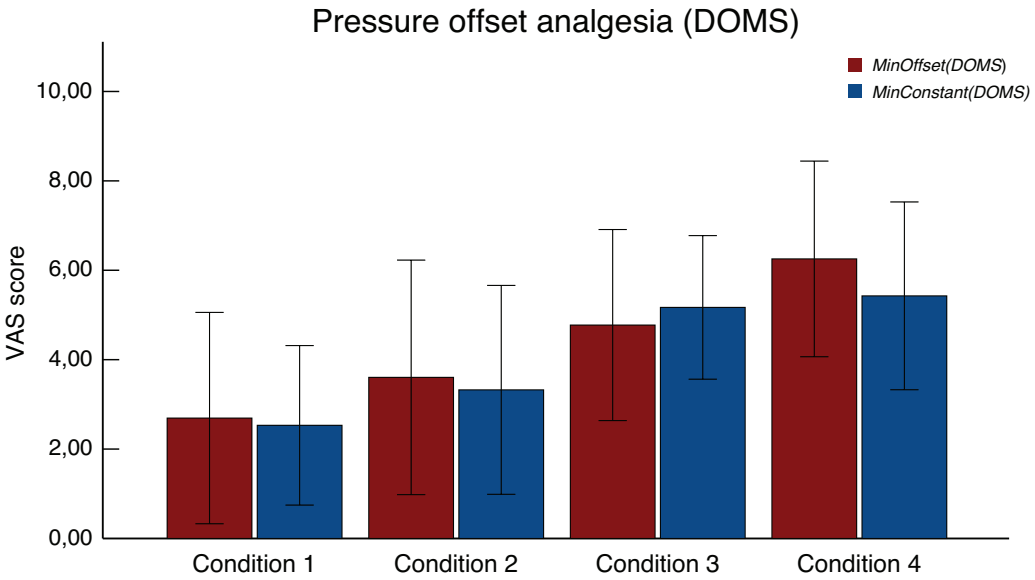

Figure 7.8: *Mean and standard deviations of the parameters MinOffset(DOMS) and MinConstant(DOMS).*

The rmANOVA was used to test the hypothesis that there is a statistical significant difference between *MinOffset(DOMS)* and *MinConstant*, hence if *MinOffset(DOMS)* is significant smaller this would indicate that OA exists during noxious pressure stimuli. The rmANOVA shows that there is no statistical significant difference between the mean of *MinOffset* and *MinConstant* ($F(3) = 1.041$, $P = 0.361$), indicating that OA does not exist during noxious pressure stimulation in subjects having DOMS.

7.4 Temporal summation

The magnitude of TS was investigated using noxious pressure stimulations. The increase in pain rating was calculated by identifying an initial and final VAS score in the interval described in 6.3 on page 21. The mean initial and final values from pressure A and B with and without DOMS are listed in Table 7.8 with corresponding standard deviations.

Mean values and SD	
Pressure A initial	3.26 ± 1.7
Pressure A final	4.13 ± 2.12
Pressure B initial	4.05 ± 2.13
Pressure B final	5.59 ± 2.01
Pressure A initial(DOMS)	3.99 ± 2.36
Pressure A final(DOMS)	4.30 ± 2.29
Pressure B initial(DOMS)	5.29 ± 1.93
Pressure B final(DOMS)	5.58 ± 1.72

Table 7.8: *Mean and standard deviations of initial and final values.*

The final values in Table 7.8 are greater then the corresponding initial values. This is additionally illustrated in Figure 7.9 on the next page. The figure indicates a difference in VAS score between pressure A and pressure B and a difference in VAS scores between baseline and DOMS. A rmANOVA was applied in order to investigate if there is a statistical significant difference in mean between the initial and final values of TS and between baseline and DOMS for the two pressures.

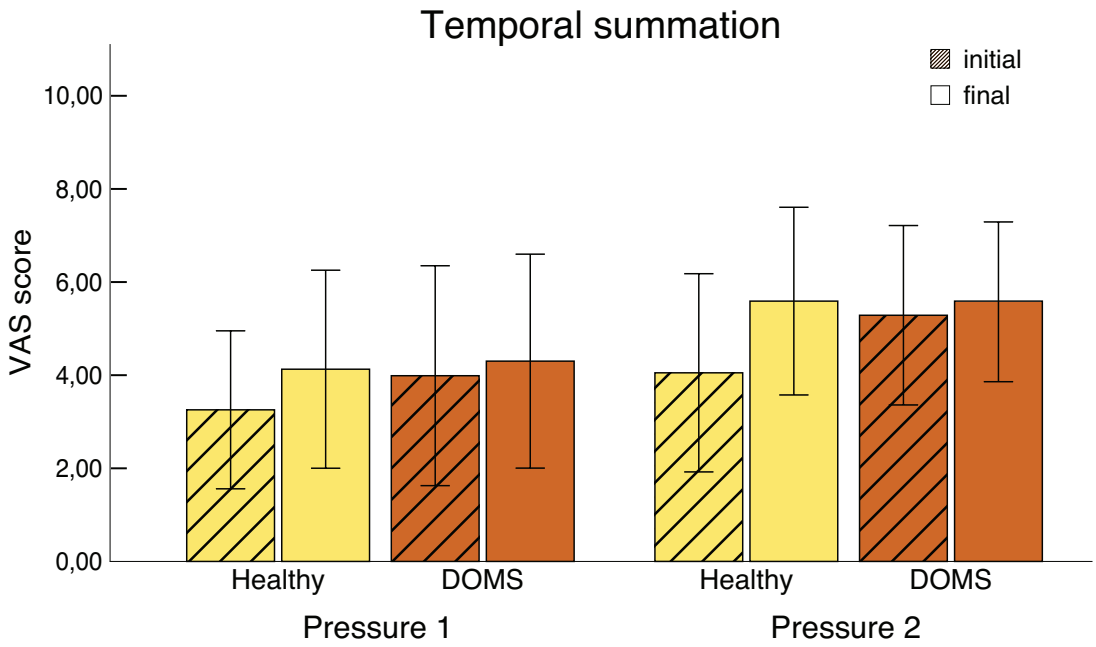

Figure 7.9: *Mean and standard deviations of initial (hatched) and final (full color) values in baseline (in yellow) and DOMS (in brown) at pressure A and pressure B in TS.*

A rmANOVA was used to test the hypothesis that the factors pressure (A,B), stimulus (initial, final) and DOMS (baseline, DOMS) could cause a statistical significant difference between VAS scores. The rmANOVA shows no statistical significant difference between the mean of initial and final VAS scores ($F(3) = 0.227$, $P = 0.750$), indicating that TS does not cause pain facilitation.

7.5 Conditioned pain modulation

The effect of CPM was investigated by comparing pressures at VAS 7 during PPT measurements as described in Section 6.4 on page 22. The mean values and the corresponding standard deviations are listed in Table 7.9.

Mean values and standard deviation	
PPT	54.2 ± 20.34
CPM	69.25 ± 20.01
PPT(DOMS)	47.03 ± 16.94
CPM (DOMS)	66.92 ± 25.11

Table 7.9: *Mean and standard deviation of PPT and CMP measures.*

The results indicate an increase in pressure between PPT and CPM at both baseline and DOMS. Figure 7.10 on the following page illustrates the mean and standard deviations.

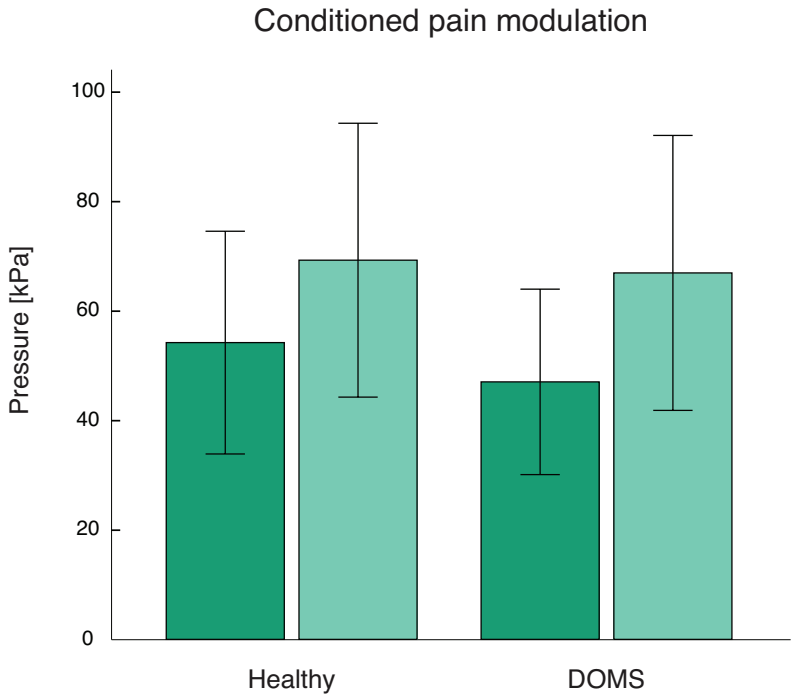

Figure 7.10: *Mean and standard deviations of pressure at VAS 7 during PPT (in dark green) and CPM (in light green).*

In order to investigate if there is a statistical significant difference between the mean values a paired student's t-test was performed. The results are given in Table 7.10. A significant difference is found between VAS 7 pressures of PPT and CPM measurements (P = 0.01) and additionally between PPT(DOMS) and CPM(DOMS) measurements (P = 0.00005).

P-values from a paired student's t-test	
PPT-CPM	0.01*
PPT(DOMS)-CPM(DOMS)	0.00005*

Table 7.10: *Results from a paired student's t-test. Differences with a significance level less than 0.05 is marked with an asterisk.*

8

Discussion

8.1 Positive results of control experiments

The present study aimed to determine whether or not it was possible to elicit offset analgesia (OA) during noxious pressure stimulations in healthy subjects and subjects experiencing delayed onset muscle soreness (DOMS). The study succeed in observing significant heat OA and to confirm temporal summation (TS) and conditioned pain modulation (CPM) as positive controls of the subject indicating no presence of chronic pain condition or central sensitization. However it was not possible to support the existence of mechanical OA as an inhibitory mechanism in noxious pressure stimulations.

Several studies have replicated the findings of Grill and Coghill [2002] by verifying the existence of OA in noxious heat stimulations [Derbyshire and Osborn, 2008, Gallez et al., 2005, Martucci et al., 2012b]. As a control to the main experiment the subjects completed the standard OA paradigm using noxious heat stimulation with three-temperature stimuli of $48-49-48\,^{\circ}\mathrm{C}$. Heat OA has been observed at temperature stimuli ranging from $45-46-45\,^{\circ}\mathrm{C}$ [Niesters et al., 2011] to $49-50-49\,^{\circ}\mathrm{C}$ [Grill and Coghill, 2002]. The control experiment showed a significant OA effect indicating, that subjects do respond to changes in the applied stimuli. This confirmed the existence of heat OA, which further supports the results obtained in previous studies observing the heat OA [Gallez et al., 2005, Grill and Coghill, 2002, Martucci et al., 2012b].

In TS no significant difference between initial and final VAS scores was observed. TS is defined as a progressive increment in pain perception during a sequence of stimuli of equal intensity. Enhancement of TS has been linked to central sensitization [Arendt-Nielsen et al., 2010, Staud et al., 2003]. Studies have found that central sensitization is partly activated by glutamate and peptides released by active nociceptors [Kellstein et al., 1990, Marvizon et al., 1997] and that it may result in the pain manifestations (e.g. referred pain) of chronic musculoskeletal disorders [Arendt-Nielsen and Graven-Nielsen, 2003]. TS has furthermore shown to be affected by gender [Sarlani et al., 2004] and anxiety [Robinson et al., 2004]. Hastie et al. [2005] formed four distinct clusters from 188 healthy subjects performing a series of pain tasks. The cluster associated with increased TS had a significantly higher percentage of females compared to males. This may explain the absence of TS in the present study as it comprises a majority of males (70 %). Noxious mechanical stimuli applied to fingers and shoulders has additionally induced an enhanced TS in subjects suffering from chronic tension-type headache (CTH) compared to healthy controls [Cathcart et al., 2010]. The absence of temporal summation in the present study indicates a normal pain facilitation systems and that the subjects were considered not to be central sensitized.

A significant difference is observed between VAS 7 in PPT measurements and VAS 7 in CPM measurements indicating that pain modulation does occur. Studies of CPM have observed an impaired CPM effect in chronic pain conditions such as fibromyalgia [Cathcart et al., 2010, Koseka and Hansson, 1997]. Thus the presence of CPM in the present study indicates that the subjects are not in a state of chronic pain. Martel et al. [2013] conducted experiments of the temporal stability of CPM in patients with chronic pain. They observed significant lower

effects of CPM in females compared to males. This supports the findings of the present study observing a significant effect of CPM. This may be duo to a majority of males (70 %) enrolled in the experiment. Furthermore Martel et al. [2013] concluded a lack of reproducibility of CPM in males but not in females. The significant effect of CPM in this study furthermore indicates normal pain inhibitory systems and the subjects are considered not to be affected by chronic pain conditions or central sensitization.

8.2 Visual analog scale

The subjects used an electronic VAS (eVAS) to rate their pain intensity. VAS is a highly subjective measure and the reliability and validity has been heavily researched, as reliable measures are necessary in pain research [Bijur et al., 2001]. Several studies have found the reliability of the VAS to be good [Gift, 1989, Jensen et al., 2003, McMahon and Koltzenburg, 2006, Sindhu and Shechtman, 2011]. The present study supports those findings, as the subjects in general are able to rate their pain according to pressure intensities. The VAS score increases as the pressure increases and vice versa. Furthermore there is a trend showing a consistency in VAS ratings between different trial at equal pressures, see Figure 8.1.

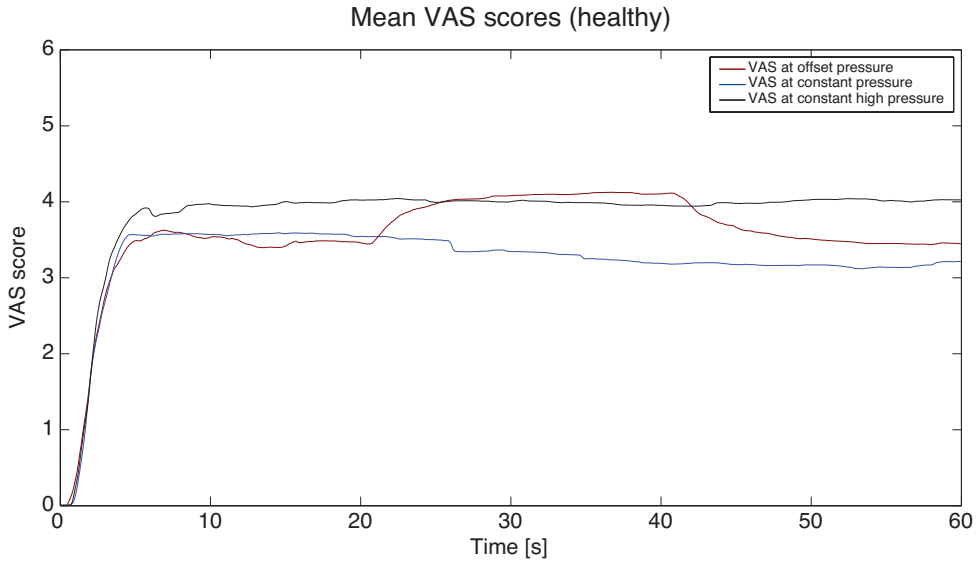

Figure 8.1: *Mean VAS scores corresponding to different pressures. There is a trend showing a consistency in VAS ratings between different trial at equal pressures.*

When subjects are exposed to a new method such as noxious pressure applied by a cuff, they need to get comfortable with the equipment in order to obtain reliable results. A training session was incorporated and an increase in PPT and PPTol were observed. However the results from the subject's PPT, PPTol and temporal summation measures were based on a single measure respectively. More reliable results might have been available if a mean of additional measures were obtained. The applied pressures in the OA paradigm were normalized to the subject's PPT and PPTol measures which emphasizes the importance of training sessions and thus reliable VAS ratings.

It can be discussed whether the subjects have understood the VAS: what the pain threshold and pain tolerance is. Some subjects terminated the stimulations before reaching a VAS 10 rating. This might affect the results, as the noxious pressures are normalized to the individual subject's pain levels. Thus, if the tolerance is less than the actual tolerance level, all pressures will be lower and some might not be noxious, which is an assumption that has to be satisfied when investigating OA.

As the subjects are able to rate their pain on a eVAS and the VAS is a valid measure, the absence of OA in muscle pain is concluded not to be a result of faulty ratings in VAS, which was one of the main considerations when first applying a subjective measure.

8.3 Absence of offset analgesia

Despite verification of OA in noxious heat stimulation and the positive control of TS and CPM it was not possible to confirm OA as an analgesic response evoked by noxious pressure stimulations. The present study was designed in favor of a potential observation of mechanical OA. To our knowledge, no studies investigating mechanical OA have been conducted. The exact time of interest for observing mechanical OA are therefore unknown. The analysis was constructed to provide OA with the best conditions. This was done by creating algorithms detecting the minimum VAS score in P3 and thus yielding the most distinct effect of OA.

Likewise is was unknown at which pressure intensity OA was most pronounced. Studies of heat OA has been conducted at a variety of temperatures; $41 - 42 - 41\,^{\circ}C$ [Derbyshire and Osborn, 2009], $45 - 46 - 45\,^{\circ}C$ [Niesters et al., 2011, Nilsson et al., 2013], $48 - 50 - 48\,^{\circ}C$ [Grill and Coghill, 2002, Yelle et al., 2008b] and $49 - 50 - 49\,^{\circ}C$ [Grill and Coghill, 2002, Martucci et al., 2012b, Yelle et al., 2009]. Additionally the temperatures used to observe heat OA has been determined by measures of pain tolerance thresholds (PTTs) and rating on the Gracely intensity scale [Derbyshire and Osborn, 2008, Nilsson et al., 2013]. Accordingly, in this study it was decided to perform the mechanical OA at four different pressures normalized to PPT and PPTol measurements. In this way the experiment has provided the best conditions for OA to be observed. Despite the effort of performing the experiment at different pressures and furthermore applying an algorithm favoring detection of the analgesic responses, this failed. The fact that the experiment design has provided mechanical OA with the best conditions strengthens the absence of OA induce by noxious pressure stimulations.

8.4 Temporal sharpening

Grill and Coghill [2002] suggested post-stimulus inhibition as a temporal contrast enhancement mechanism with the purpose of amplifying the perception of stimulus energy decreases. OA, as observed during noxious heat stimulations, was proposed to reflect such a mechanism [Grill and Coghill, 2002]. This was supported by Martucci et al. [2012b] describing OA as a temporal sharpening filter. The contention of a temporal contrast enhancement mechanism in heat may be supported by characteristics of the heat modality. Changes in temperature is not supported by all modalities of the sensory system. Contrary temperature detection, detection of deep muscle pain resulting from e.g strikes or long lasting compressions can be supported by vision and touch. This may give rise to a specific temporal contrast enhancement mechanism of the temperature modality and furthermore it clarifies the absence of this mechanism within the touch modality. However modulations of temporal integration in somatosensory information has been observed [Gabernet et al., 2005].

8.5 Reperfusion injuries and mechano-insensitive nociceptors

A pneumatic tourniquet cuff was used to apply noxious pressures in this study. Thus, ischaemia reperfusion injuries may affect the results. Reperfusion injuries occur when blood supply is restored in an ischemic organ or tissue [Carden and Granger, 2000]. The restoration of blood supply when the cuff is deflated can cause micovascular injuries affecting e.g. skeletal muscles and nerves resulting in muscle dysfunction and a burning sensation [Estebe et al., 2011]. If the subjects experience reperfusion pain, this might complicate the results, as it would mask

the actual pain relieve that would occur when the pressure is slightly decreased. A study by Tuncali et al. [2006] states that the tourniquet pressure must be 75-100 mmHg greater than the systolic arterial pressure in order to occlude arterial blood. Thus, the P3 pressure applied during OA paradigms must be less than 26-29.3 kPa to cause reperfusion pain. The P3 pressure was in between this interval for 29 % of the subjects. Thus, reperfusion pain may have masked a potential pain relief following a decrease in noxious pressure stimulations.

Another phenomenon which might explain the absence of OA during deep muscle pain is mechano-insensitive nociceptors. C nociceptors can be divided into mechano-responsive and mechano-insensitive nociceptors [Schmidt et al., 1995]. Several studies have shown that mechano-insensitive nociceptors are unresponsive to mechanical stimuli, but become responsive in accordance with inflammation and some do respond to noxious heat stimuli [Schmelz et al., 1997, Schmidt et al., 1995, Weidner et al., 1997]. The mechano-insensitive C nociceptors accounts for approximately 30 % of C nociceptors [Schmidt et al., 1995], and it can be hypothesized that the noxious pressures applied in the present study did not activate these nociceptors. However the noxious heat stimuli used in the present control experiment and the original OA paradigm described by [Grill and Coghill, 2002], might have activated the mechano-insensitive nociceptors causing a different pain sensation. The pain model created by DOMS was applied in order to active these mechano-insensitive C fibers. Nevertheless no significant difference was observed between mechanical OA trials with and without DOMS.

8.6 Methodological considerations

The stimulation duration of P1, P2 and P3 applied during the mechanical OA trials of this study was based on a pilot study. In the first part of the pilot study four subjects were tested using the stimulation duration (8s-8s-8s). At the end of P3 stimulations the VAS scores were still decreasing indicating that the subjects needed longer stimulation durations to stabilize the pain ratings. Another test paradigm (20s-20s-20s) was applied and by comparing the results, this setup provided more stable results. However other studies investigating OA with noxious heat stimulations have used the stimulation duration (5s-5s-20s) [Derbyshire and Osborn, 2008, Gallez et al., 2005, Grill and Coghill, 2002]. Thus, instead of prolonging the stimulation duration of P1, P2 and P3, only prolonging the stimulation duration of P3 might have provided results resembling those of [Grill and Coghill, 2002]. By prolonging all pressure stimulations the possibility of adaptation of the primary afferents might increase and the detection of OA would thereby decrease.

Pressure stimulations were mainly applied with a double chambered tourniquet cuff (13 cm) placed around the calf muscles of the dominant leg. Furthermore a single chambered cuff (7.5 cm) placed around the contralateral upper arm and one of the chambers in the double chambered cuff were used in the assessment of CPM. When measuring the effect of CPM, PPTs conducted with the double chambered cuff were compared with the CPM PPTs conducted with the pressure delivered through only one chamber of the cuff. A study by Polianskis et al. [2002] found that PPT and PPTol increase when using a single chambered cuff compared with a double chambered cuff. Thus, when comparing PPT values with CPM PPT values of this study the CPM PPT value might be to high, resulting in a better functioning inhibitory system.

In connection with these observations a fundamental difference between the heat and the pressure experiment has been noticed. The mean VAS scores through out the experiments appear to be different in magnitude. The pain ratings during the experiment applying noxious pressure stimulations seem considerably lower than those of the control experiment applying noxious heat stimulations. In a study by Derbyshire and Osborn [2009] OA was investigated using lower heat stimulations ($41 - 42 - 41\,^{\circ}\mathrm{C}$) than comparable studies [Gallez et al., 2005, Grill and Coghill, 2002]. No significant difference was found between heat OA trials and heat constant

trials [Derbyshire and Osborn, 2009]. The pressure stimulations in the present study may be comparable to the low heat stimulations in the study of Derbyshire and Osborn [2009], which may have caused the absence of OA. Thus, the pressure intensities may influence the magnitude of OA and therefore has to be taken into consideration.

9

Conclusion

The aim of this study was to investigate the existence of offset analgesia (OA) in deep muscle pain evoked by noxious pressure stimulations and furthermore to evaluated whether OA is affected by central acting mechanisms and sensitized nociceptors.

The study succeeded in observing significant heat OA and to confirm the pain modulating mechanisms temporal summation (TS) and conditioned pain modulation (CPM) as positive controls of the subject indicating no presence of chronic pain conditions or central sensitization. The study applied noxious pressure stimuli to the calf muscle using a double chambered pneumatic tourniquet cuff in order to evoke OA. Four different pressure paradigms were investigated in healthy subjects before and after applying a pain model with delayed onset muscle soreness (DOMS). No significant results was found towards the existence of OA in deep muscle pain.

Within the limitations of this study the hypothesis of OA as an endogenous analgesic mechanism evoked in deep muscle pain by noxious pressure stimuli cannot be supported. It is suggested that the absence of OA in noxious pressure stimulations is due to lack of temporal contrast enhancement in the submodality pressure. Additional research is recommended to gain more knowledge of OA as a pain modulating mechanism in deep muscle pain.

A

Pilot study

Prior to the main experiment a pilot study was conducted in order to test whether it was possible to elicit offset analgesia (OA) by applying noxious pressure stimuli to calf muscles and to test the effect of different setup parameters.

During the first part of the pilot study five pressures were tested as the lower pressure of the OA paradigm.The pressures were determined from the PPT-PPTol interval (Figure A.1). PPT corresponded to 0 % of the interval and PPTol was equal to 100 %. The five low pressures corresponded to 30 %, 40 %, 50 %, 60 % and 70 % respectively. The upper pressure was defined as the subject's PPTol (see Figure 5.2 on page 15).

The stimulation consists of three pressure stimuli:

- A constant upper pressure (8 s duration)
- A constant lower pressure (8 s duration)
- First a upper pressure (8 s duration) and then an incremental decrease to the lower pressure (8 s duration)

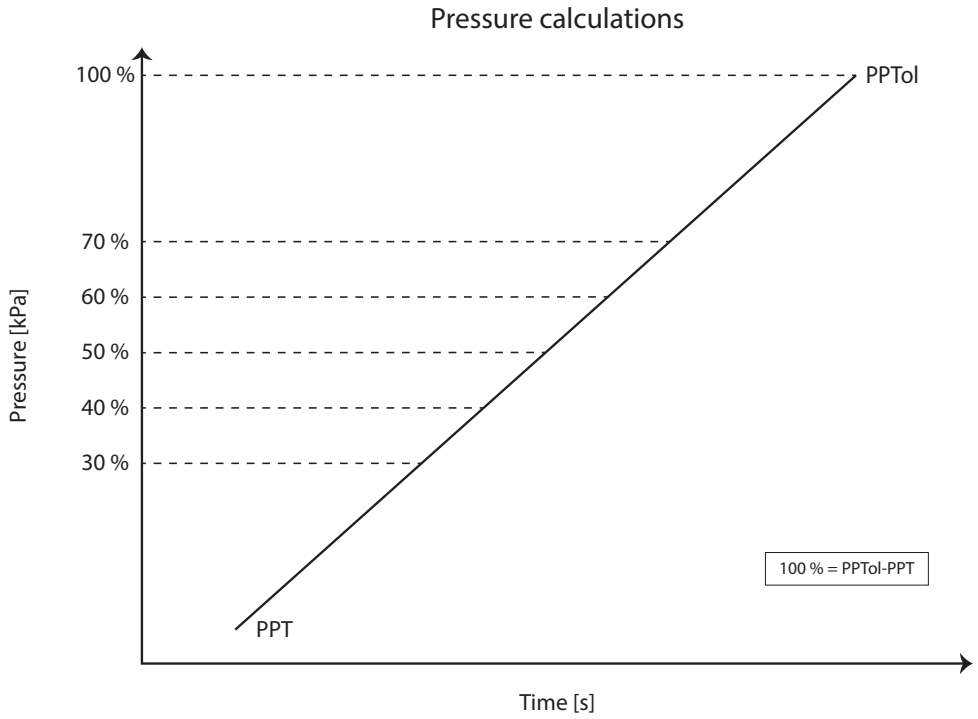

Figure A.1: *The different pressures used in the first part of the pilot study.*

As the first configuration did not show any trend of elicited OA within the four subject, a new setup was tested. This included three pressure: P1, P2 and P3 (see Figure A.2 on the next page)

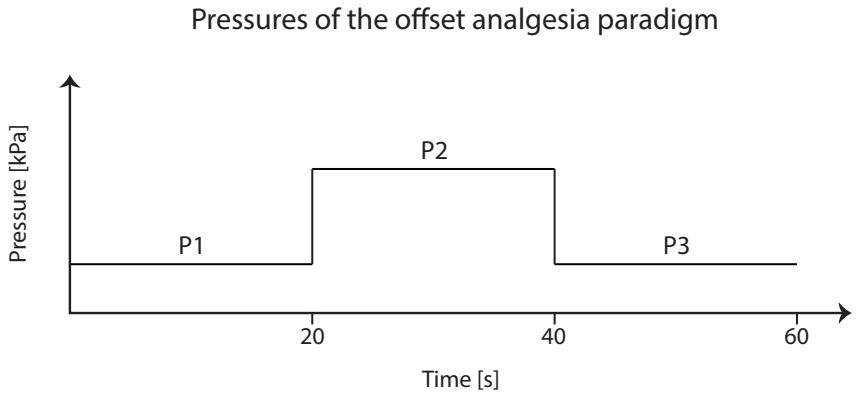

Figure A.2: *Illustration of the pressures in the OA paradigm.*

One of the main changes was the upper pressure. During part 1 the upper pressure was set to the subject's PPTol, and this might have been to painful to discriminate the pain intensity experienced within the different pressures. Part 2 of the pilot study therefore included four pressures, which were used as lower pressures and two pressures used as upper pressures. The applied pressures were normalized to the individual subject's PPT and PPTol. Furthermore the pressure of part 2 of the pilot study was determined from the PPT-PPTol interval (Figure A.3). PPT corresponded to 0 % of the interval and PPTol was equal to 100 %. The two high pressures were defined as A = 50 % and B = 75 %. The four low pressures A1, A2, B1 and B2 were defined as 30 %, 40 %, 55 % and 65 % respectively, see Figure A.3.

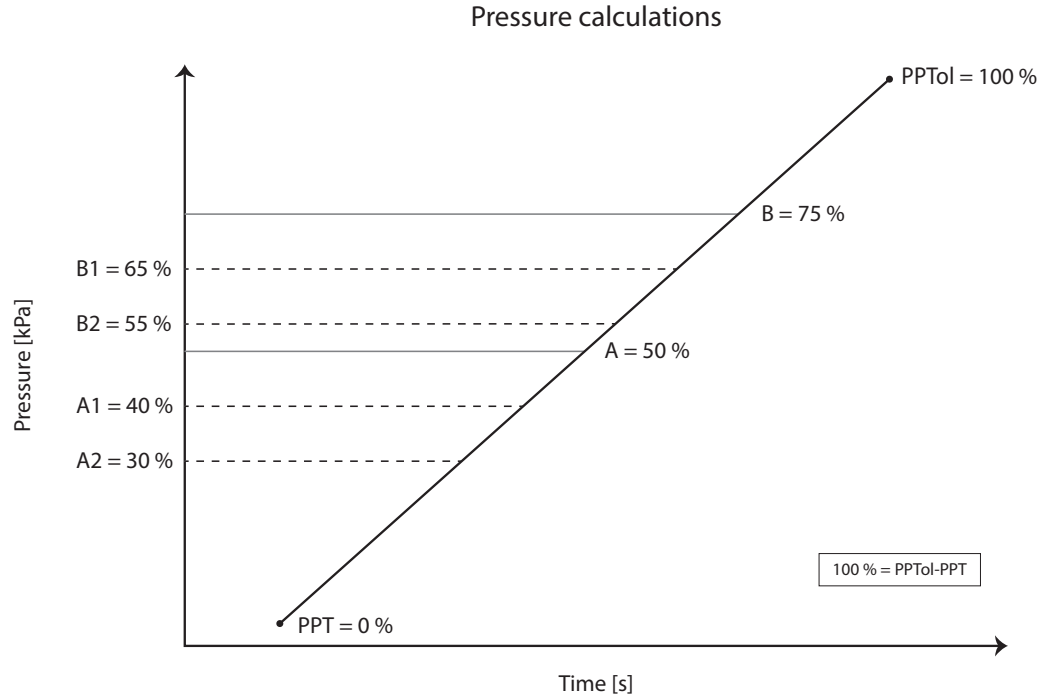

Figure A.3: *Definitions of the different pressures applied during the mechanical stimulations.*

Additionally the stimulation duration was changed. In part 1 it seemed too short, as subject needed a couple of seconds to evaluate the pain intensity on the VAS for the different pressures. Furthermore the stimulation duration of the constant pressures were no the same as the stimulation duration of the OA paradigm, which could lead to wrong interpretations due to adaptation of primary afferents.

The stimulation consisted of three pressure stimuli:

- A constant upper pressure (60 s duration)

- A constant lower pressure (60 s duration)
- A lower pressure P1 (20 s duration) then an incremental increase to the upper pressure P2 (20 s duration) followed by an incremental decrease to the initial pressure P3 (20 s duration)

In order to determine if OA was present minimum VAS scores were compared between the constant lower pressures and the pressure during the P3 stimulation. This was done in order to observe whether the minimum VAS score of P3 was lower than the minimum VAS score of a constant pressure at the same level in corresponding time interval.

Furthermore the different parts of the experimental protocol were tested on the investigators with the intention to clarify the process, optimize data collection and make the experiment as inconvenient as possible for future subjects.

B

Experiment protocol

This protocol was applied under the project title *Evaluation of offset analgesia in muscle pain* in April 2014 by Pernille Brøndum and Michael Holt supervised by Carsten Dahl Mørch and Kristian Kjær Petersen (Aalborg University, Denmark).

The experiment was conducted at:
Department of Health, Science and Technology, Aalborg University
Fredrik Bajers Vej 7, 9220 Aalborg, Denmark

Subjects

The aim of the experiment was to investigate the existence of offset analgesia (OA) in muscle pain. The subjects had to meet certain inclusion and exclusion criteria describing their individual condition. The inclusion and exclusion criteria are listed below:

Inclusion criteria

- Healthy men and women
- 18-60 years

Exclusion criteria

- Participating women must not be pregnant
- Drug addiction, defined as the use of cannabis, opioids or other drugs
- Current use of medications that may affect the experiment (pain relievers, psychotropic drugs, etc.)
- Previous or current neurological diseases
- Lack of interpersonal skills
- Consumption of alcohol, caffeine, nicotine or painkillers 24 hours before the study day
- Acute or chronic pain
- Tattoos or moles in the stimulation area
- Participation in other experiments 1 week before the study day and in parallel with the study

Apparatus and instrumentation

- A 30x30 mm thermode with rise and fall rate of $6\,^{\circ}\mathrm{C}/s$ (Medoc, Ramat Yishai, Israel).
- Medoc Pathway pain and sensory evaluation system (Medoc, Ramat Yishai, Israel).
- A double chambered 13 cm wide pneumatic tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany).
- A single chambered 7.5 cm wide pneumatic tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany).
- A computer-controlled air-compressor (NociTech Aps, Denmark)
- Cuff Control LabView5 (National Instruments, Austin, Texas, U.S.) for controlling cuff pressures.

- A 10 cm electronic visual analog scale (eVAS) (Aalborg University, Denmark) with the extremes 'no pain' and 'worst imaginable pain')[Price et al., 1994].

Experiment sessions

The experiment is divided into three sessions.

- Day 1
  - Offset analgesia with noxious heat stimulation
- Day 2
  - Training
  - PPT/PPTol
  - Offset analgesia with noxious pressure stimuli
  - Temporal summation
  - CPM
  - DOMS
- Day 3
  - PPT/PPTol
  - Offset analgesia with noxious pressure stimuli
  - Temporal summation
  - CPM

Experiment procedures

Prior to the experiments written informed consents are provided to all subjects participating in this study. Furthermore they are given a written advisement of the eVAS to ensure a consistent use of the measure and to make sure that the subjects are comfortable using the eVAS.

Offset analgesia with noxious heat stimulations

Thermal stimulations are applied to the ventral forearm. The thermode is fixed to the dominant forearm 5 cm distal to the elbow joint with a velcro strap. Training trials are completed on the non-dominant arm to ensure that the subject are familiarized with the applied temperature and the use of the eVAS. The following stimulations is presented in a randomized order:

- A constant noxious heat simulation of 48 °C/s (30 s duration). The subjects continuously rate the pain intensity on a eVAS
- Three contiguous phases: an initial temperature stimulus (T1, 48 °C), an incremental increase to a second temperature stimulus (T2, 49 °C) followed by a decrease to a third temperature stimulus (T3, 48 °C)[Grill and Coghill, 2002]. The subjects continuously rate the pain intensity on a eVAS.

The procedures of the training trials described above are reapplied in the measures of OA.

Training for pressure stimulations

Unless otherwise stated the double chambered cuff is placed around the shin of the dominate leg at the level of the belly of the gastrocnemius-soleus muscles in accordance with Polianskis et al. [2001]. Moreover the subject is wearing earmuffs and is rested in a comfortable position on a couch with an elevated headboard during the assessment.

Training trials were performed in order to ensure that the subject are comfortable with the applied pressure and the use of the eVAS. Subjects were trained to rate their perceived pain intensity using the eVAS. The training consists of of the following pressure stimulations:

- A PPT/PPTol measurement is performed. The cuff is inflated at a compression rate of 1kPa/s and the subject continuously rate the pain intensity on the eVAS and presses a hand-held release button when the tolerance level is reached.
- A constant pressure is applied for 60 s at a pressure corresponding to a VAS 5 rating of the first PPT/PPTol measurement and the subject continuously rates the pain intensity on the eVAS.
- The cuff is inflated at a compression rate of 1 kPa/s and the subject continuously rates the pain intensity on a VAS and presses a hand-held release button when the tolerance level is reached.
- Three contiguous pressures are applied: A constant pressure (20 s duration) is applied at a pressure corresponding to a VAS 4 rating of the second PPT/PPTol measurement. Following this the pressure increases to a second pressure corresponding to a VAS 6 rating of the second PPT/PPTol (20 s duration). Finally the pressure returns to the initial pressure (20 s duration). During this the subject continuously rates the pain intensity on the eVAS.

Pressure pain threshold and pressure pain tolerance

The pressure pain threshold (PPT) and the pressure pain tolerance (PPTol) are assessed using the following procedures:

1. The cuff is mounted.
2. The subject is instructed to rate the pain intensity continuously on the eVAS from the first sensation of pain and then press the release button when the tolerance level is reached.
3. The RAMP function is launched and the cuff is inflated.
4. When the tolerance level is reached an algorithm reads the PPT and the PPTol of the data.

Offset analgesia with noxious pressure stimuli

In order to test the existence of offset analgesia in muscle pain the function CUSTOM in *CuffControl* is used. Three different trials (illustrated in Figure B.1) are measured with varying pressures.

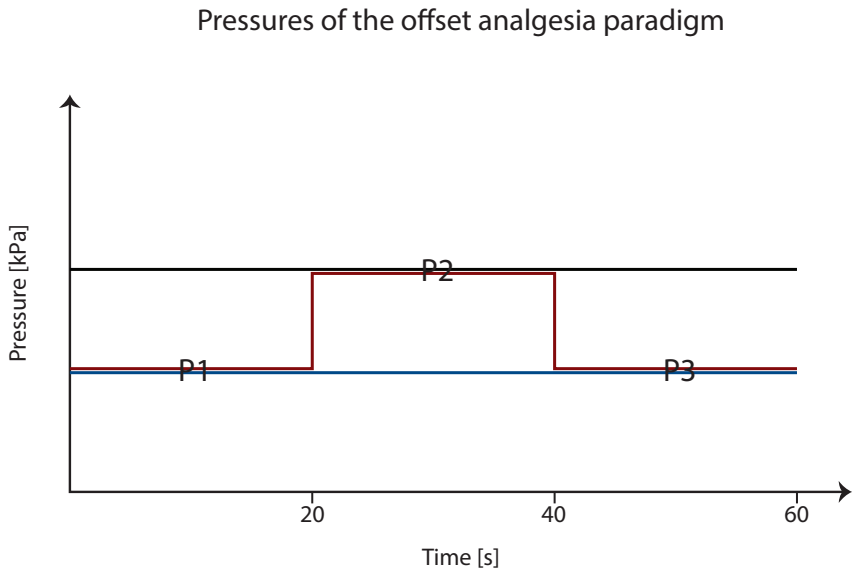

Figure B.1: *Illustration of the three trials: Offset analgesia (red) (changing pressure), constant lower pressure (blue) and constant upper pressure (black).*

The required pressure are defined from the PPT/PPTol measurement. PPT is equal to 0 % and PPTol is equal to 100 %

- Pressure A = 50 %
- Pressure B = 75 %
- Pressure A1 = 30 %
- Pressure A2 = 40 %
- Pressure B1 = 55 %
- Pressure B2 = 65 %

The subject is tested using four different sets of pressure trials. Each mechanical OA trial consists of three contiguous phases: an initial pressure (lower pressure A1, A2, B1 or B2), an increase to a second pressure (upper pressure A or B), and a decrease to a third pressure stimulus equal to the initial pressure (lower pressure A1, A2, B1 or B2). Each pressure has a duration of 20 s. Furthermore constant pressure trials of 60 s are performed. These trials are conducted with lower and upper pressures respectively. The subject continuously rates the pain intensity on an eVAS during each trial.

The recordings are grouped in four conditions. During the pressure stimulations the subject is instructed to rate the pain intensity continuously on the eVAS. Pressures and trials are randomized and breaks of 60 s between trials are applied. The procedures of noxious pressure stimulations are described in the following itemize.

- Constant pressure trials corresponding to pressure A1, A2, B1 or B2 is recorded for 60 s.
- Constant pressure trials corresponding to pressure A or B is recorded for 60 s.
- Trials of three contiguous phases: an initial pressure (pressure A1, A2, B1 or B2), an increase to a second pressure (pressure A or B), and a decrease to a third pressure equal to the initial pressure is recorded. Each pressure has a duration of 20 s.

Temporal summation

In order to measure temporal summation (TS) the function REPETITIVE in *CuffControl* is used. The applied pressures corresponding to A and B are randomized.

1. The subject is instructed only to rate the pain intensity of the pressure stimuli on the eVAS.
2. The stimulations consisted of ten pressure stimuli (1 s duration and 1 s interstimulus interval). The pressure stimuli are applied at an intensity corresponding to pressure A or B and the intensity of the interstimulus is 5 kPa.

Conditioned pain modulation

In order to measure conditioned pain modulation (CPM) the function CUSTOM in *CuffControl* is used. A single chambered cuff is placed around the contralateral upper arm at the belly of m. biceps brachii.

1. The subject is instructed to only rate the pain sensation in the arm continuously on the eVAS, from the first sensation of pain and then press the release button when their tolerance level is reached.
2. An increasing pressure with a compression rate of 1 kPa/s is applied to the cuff placed around the upper arm in order to obtain a pressure corresponding to a VAS 5 rating of the VAS.
3. The subject is instructed only to rate the pain sensation in the shin on the eVAS, from the first sensation of pain and then press the release button when their tolerance level is reached.

4. A constant pressure corresponding to a VAS 5 rating is applied to the cuff placed around the upper arm. Simultaneously, one chamber in the cuff placed around the shin is inflated with a compression rate of 1 kPa/s and a PPT/PPTol measurement is performed.

DOMS

Physical exercise is used to induce DOMS. In order to target the gastrocnemius-soleus muscles, the subject performs 10 sets of body weighted standing calf raises.

1. The subject is positioned with toes and ball of their dominant foot on a calf block.
2. Flexion and extension of the ankle is performed at 0.5 Hz. The first 8 sets consist of 20 repetitions and the last two sets of 40 repetitions. Each set is separated by 3 min.

The subject is instructed to perform as many sets as possible until exhaustion is reached.

References

L. Arendt-Nielsen and T. Graven-Nielsen. Translational aspects of musculoskeletal pain: from animals to patients. In T Graven-Nielsen, L Arendt-Nielsen, and S Mense, redaktører, *Fundamentals of Musculoskeletal Pain*, pages 347–366. IASP Press, Seattle, 2004.

Arendt-Nielsen, Brennum, Sindrup, and Bak, **1994**. L. Arendt-Nielsen, J. Brennum, S. Sindrup, and P Bak. *Electrophysiological and psychophysical quantification of temporal summation in the human nociceptive system.* Eur J Appl Physiol Occup Physiol, 68, 266–273, 1994.

Arendt-Nielsen and Graven-Nielsen, **2003**. Lars Arendt-Nielsen and Thomas Graven-Nielsen. *Central Sensitization in Fibromyalgia and Other Musculoskeletal Disorders.* Current Pain and Headache Reports, 7, 355–361, 2003.

Arendt-Nielsen and Petersen-Felix, **1995**. Lars Arendt-Nielsen and S. Petersen-Felix. *Wind-up and neuroplasticity: is there a correlation to clinical pain?* Eur. J. Anaesthesiol, pages 1–7, 1995.

Arendt-Nielsen and Yarnitsky, **2009**. Lars Arendt-Nielsen and David Yarnitsky. *Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera.* The journal of pain : official journal of the American Pain Society, 10, 556–572, 2009.

Arendt-Nielsen, Nie, Laursen, Laursen, Madeleine, Simonsen, and Graven-Nielsen, **2010**. Lars Arendt-Nielsen, Hongling Nie, Mogens B. Laursen, Birgitte S. Laursen, Pascal Madeleine, Ole H. Simonsen, and Thomas Graven-Nielsen. *Sensitization in patients with painful knee osteoarthritis.* Pain, 149, 573–581, 2010.

Bars, Villanueva, Bouhassira, and Willer, **1992**. D Le Bars, L Villanueva, D Bouhassira, and J C Willer. *Diffuse noxious inhibitory controls (DNIC) in animals and in man.* pages 55–65, 1992.

Bijur, Silver, and Gallagher, **2001**. Polly E. Bijur, Wendy Silver, and E. John Gallagher. *Reliability of the Visual Analog Scale for Measurement of Acute Pain.* Academic Emergency Medicine, 8, 1153–1157, 2001.

Carden and Granger, **2000**. Donna L. Carden and D. Neil Granger. *Pathophysiology of ischaemia-reperfusion injury.* Journal of Pathology, 190, 255–266, 2000.

Cathcart, Winefield, Lushington, and Rolan, **2010**. Stuart Cathcart, Anthony H. Winefield, Kurt Lushington, and Paul Rolan. *Noxious inhibition of temporal summation is impaired in chronic tension-type headache.* The Journal of Pain, 50, 403–412, 2010.

Cheung, Hume, and Maxwell, **2003**. K Cheung, P Hume, and L Maxwell. *Delayed onset muscle soreness: treatment strategies and performance factors.* Sports Med, 33, 145–164, 2003.

Cleak and Eston, **1992**. MJ Cleak and RG Eston. *Delayed onset muscle soreness: mechanisms and management.* Journal of Sports Science, 10, 325–341, 1992.

Coronado, Simon, Valencia, and George, **2014**. R A Coronado, C B Simon, C Valencia, and S Z George. *Experimental pain responses support peripheral and central sensitization in patients with unilateral shoulder pain.* Clinical Journal of Pain, 30, 143–151, 2014.

Defrin, Ronat, Ravid, and Peretz, **2003**. Ruth Defrin, Amit Ronat, Arnon Ravid, and Chava Peretz. *Spatial summation of pressure pain: effect of body region.* Pain, 106, 471–480, 2003.

Derbyshire and Osborn, **2009**. S. W. G. Derbyshire and J. Osborn. *Offset analgesia is mediated by activation in the region of the periaqueductal grey and rostral ventromedial medulla.* NeuroImage, 47, 1002Ð1006, 2009.

Derbyshire and Osborn, **2008**. S.W.G. Derbyshire and J. Osborn. *Enhancement of offset analgesia during sequential testing.* European Journal of Pain, 12, 980–989, 2008.

Estebe, Davies, and Richebe, **2011**. Jean-Pierre Estebe, Joanna M. Davies, and Philippe Richebe. *The pneumatic tourniquet: mechanical, ischaemia-reperfusion and systemic effects.* Eur J Anaesthesiol, 28, 404–411, 2011.

Fields, **2004**. Howard Fields. *State-dependent opioid control of pain.* Neuroscience, 5, 565–575, 2004.

Friden, Sjostrom, and Ekblom, **1984**. J Friden, M Sjostrom, and B Ekblom. *Myofibrillar damage following intese eccentric exercise in men.* International Journal of Sports Medicine, 4, 170–176, 1984.

Gabernet, Jadhav, Feldman, Carandini, and Scanziani, **2005**. Laetitia Gabernet, Shantanu P. Jadhav, Daniel E. Feldman, Matteo Carandini, and Massimo Scanziani. *Somatosensory integration controlled by dynamic thalamocortical feed-forward inhibition.* Neuron, 48, 315–327, 2005.

Gallez, Albanese, Rainville, and Duncan, **2005**. Ariane Gallez, Marie-Claire Albanese, Pierre Rainville, and Gary H. Duncan. *Attenuation of sensory and affective responses to heat pain: Evidence for contralateral mechanisms.* Journal of Neurophysiology, 94, 3509–515, 2005.

Gebhart, Sandkuhler, Thalhammer, and Zimmermann, **1983**. G. F. Gebhart, J. Sandkuhler, J. G. Thalhammer, and M. Zimmermann. *Inhibition of spinal nociceptive information by stimulation in midbrain of the cat is blocked by lidocaine microinjected in nucleus raphe magnus and medullary reticular formation.* Journal of Neuropysiology, 50, 1446–1459, 1983.

Gerhart, Yezierski, Giesler, and Willis, **1981**. KD Gerhart, RP Yezierski, GJ Jr Giesler, and WD Willis. *Inhibitory receptive fields of primate spinothalamic tract cells.* J neurophysiol, 46, 1309–1325, 1981.

Gift, **1989**. Audrey G. Gift. *Visual Analog Scales: Measurement of subjective phenomena.* Nurs Res, 38, 286–288, 1989.

Graven-Nielsen, Arendt-Nielsen, Svensson, and Jensen, **1997**. T. Graven-Nielsen, L. Arendt-Nielsen, P. Svensson, and T.S. Jensen. *Stimulus-response functions in areas with experimentally induced referred muscle pain - a psychophysical study.* Brain research, 774, 121–128, 1997.

Graven-Nielsen, Aspegren, Henriksson, Bengtsson, Sorensen, Johnson, Gerdle, and Arendt-Nielsen, **2000**. T. Graven-Nielsen, K.S. Aspegren, K.G. Henriksson, M. Bengtsson, J. Sorensen, A. Johnson, B. Gerdle, and L. Arendt-Nielsen. *Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients.* Pain, 85, 483–491, 2000.

Graven-Nielsen, Wodehouse, Langford, Arendt-Nielsen, and Kidd, **2012**. T. Graven-Nielsen, T. Wodehouse, R.M. Langford, L. Arendt-Nielsen, and B.L. Kidd. *Normalization of Widespread Hyperesthesia and Facilitated Spatial Summation of Deep-Tissue Pain in Knee Osteoarthritis Patients After Knee Replacement.* Arthritis & Rheumatology, 64, 2907–2916, 2012.

Thomas Graven-Nielsen and Lars Arendt-Nielsen. Human models and clinical manifestations of musculoskeletal pain and pain-motor interactions. In *T Graven-Nielsen, L Arendt-Nielsen & S Mense (red), Fundamentals of Musculoskeletal Pain. IASP Press*, pages 153–183. 2008.

Graven-Nielsen and Arendt-Nielsen, **2010**. Thomas Graven-Nielsen and Lars Arendt-Nielsen. *Assessment of mechanisms in localized and widespread musculoskeletal pain.* Nature reviews Rheumatology, 6, 599–606, 2010.

Graven-Nielsen, Mense, and Arendt-Nielsen, **2004**. Thomas Graven-Nielsen, Siegfried Mense, and Lars Arendt-Nielsen. *Painful and non-painful pressure sensations from human skeletal muscle.* Experimental Brain Research, 159, 273–283, 2004.

Grill and Coghill, **2002**. Joshua D. Grill and Robert C. Coghill. *Transient analgesia evoked by noxious stimulus offset.* The American Physiological Society, 87, 2205–2208, 2002.

Hastie, III, Robinson, Glover, Campbell, Staud, and Fillingim, **2005**. Barbara A. Hastie, Joseph L. Riley III, Michael E. Robinson, Toni Glover, Claudia M. Campbell, Roland Staud, and Roger B. Fillingim. *Cluster analysis of multiple experimental pain modalities.* Pain, 116, 227–237, 2005.

Hough, **1902**. Theodore Hough. *Ergographic studies in muscular soreness.* Am J Physiol, 7, 76–92, 1902.

IASP, **May 2012**. IASP. *The International Association for the Study of Pain*, 2012. URL http://www.iasp-pain.org.

Jensen, Chen, and Brugger, **2003**. Mark P. Jensen, Connie Chen, and Andrew M. Brugger. *Interpretation of Visual Analog Scale Ratings and Change Scores: A Reanalysis of Two Clinical Trials of Postoperative Pain.* The Journal of Pain, 4, 407–414, 2003.

Jensen-Dahm, Werner, B. Dahl, Jensen, Ballegaard, Hejl, and Waldemar, **2014**. Christina Jensen-Dahm, Mads U. Werner, Jørgen B. Dahl, Troels Staehelin Jensen, Martin Ballegaard, Anne-Mette Hejl, and Gunhild Waldemar. *Quantitative sensory testing and pain tolerance in patients with mild to moderate Alzheimer disease compared to healthy control subjects.* Pain, 2014.

Jespersen, Amris, Graven-Nielsen, Arendt-Nielsen, Bartels, Torp-Pedersen, Bliddal, and Danneskiold-Samsoe, **2013**. A Jespersen, K Amris, T Graven-Nielsen, L Arendt-Nielsen, E M Bartels, S Torp-Pedersen, H Bliddal, and B Danneskiold-Samsoe. *Assessment of pressure-pain thresholds and central sensitization of pain in lateral epicondylalgia.* Pain Med, 14, 297–304, 2013.

Kanda, Sugama, Hayashida, Sakuma, Kawakami, Miura, Yoshioka, Mori, and Suzuki, **2013**. Kazue Kanda, Kaoru Sugama, Harumi Hayashida, Jun Sakuma, Yasuo Kawakami, Shigeki Miura, Hiroshi Yoshioka, Yuichi Mori, and Katsuhiko Suzuki. *Eccentric exercise-induced delayed-onset muscle soreness and changesin markers of muscle damage and inflammation.* Exerc Immunol Rev, 19, 72–85, 2013.

Kandel, Schwartz, Jessell, Siegelbaum, and Hudspeth, **2013**. Eric R. Kandel, James H. Schwartz, Thomas M. Jessell, Steven A. Siegelbaum, and A. J. Hudspeth. *Principles of neural science.* McGraw-Hill Medical, fifth edition edition, 2013.

Kellstein, Price, Hayes, and Mayer, **1990**. D E Kellstein, D D Price, R L Hayes, and D J Mayer. *Evidence that substance P selectively modulates C-fiber-evoked discharges of dorsal horn noci- ceptive neurons.* Brain Research, 526, 291–298, 1990.

Koltzenburg and Handwerker, **1994**. M. Koltzenburg and H.O. Handwerker. *Differential ability of human cutaneous nociceptors to signal mechanical pain and to produce vasodilatation.* J Neurosci, 25, 1756–1765, 1994.

Kopf and Patel, **2010**. Andreas Kopf and Nilesh B. Patel. *Guide to Pain Management in Low-Resource Settings*. International Association of the Study of Pain, 2010. Chapter 3.

Koseka and Hansson, **1997**. Eva Koseka and Per Hansson. *Modulatory influences on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (HNCS) in fibromyalgia patients and healthy subjects*. The Journal of Pain, 70, 41–51, 1997.

Le Bars, **2002**. D Le Bars. *The whole body receptive field of dorsal horn multireceptive neurones*. Brain Res Brain Res Rev, 40, 29–44, 2002.

Lemming, Graven-Nielsen, Sorensen, L, and Gerdle, **2012**. D. Lemming, T. Graven-Nielsen, J. Sorensen, L. Arendt-Nielsen L, and B. Gerdle. *Widespread pain hypersensitivity and facilitated temporal summation of deep tissue pain in whiplash associated disorder: an explorative study of women*. J Rehabil Med, 44, 648–657, 2012.

Lewis, Rice, and McNair, **2012**. Gwyn N. Lewis, David A. Rice, and Peter J. McNair. *Conditioned pain modulation in populations with chronic pain: A systematic review and meta-analysis*. The Journal of Pain, 13, 936–944, 2012.

Martel, Wasan, and Edwards, **2013**. Marc O. Martel, Ajay D. Wasan, and Robert R. Edwards. *Sex differences in the stability of conditioned pain modulation (CPM) among patients with chronic pain*. Pain Medicine, 70, 1757–1768, 2013.

Martucci, Eisenach, Tong, and Coghill, **2012a**. K. T. Martucci, J. C. Eisenach, C. Tong, and R. C. Coghill. *Opioid-independent mechanisms supporting offset analgesia and temporal sharpening of nociceptive information*. Pain, 153, 1232–1243, 2012.

Martucci, Yelle, and Coghill, **2012b**. Katherine T. Martucci, Marc D. Yelle, and Robert C. Coghill. *Differential Effects of Experimental Central Sensitization on the Time-course and Magnitude of Offset Analgesia*. Pain, 153, 463–472, 2012.

Marvizon, Martinez, Grady, Bunnett, and Mayer, **1997**. J C Marvizon, V Martinez, E F Grady, N W Bunnett, and E A Mayer. *Neurokinin 1 receptor internalization in spinal cord slices induced by dorsal root stimulation is mediated by NMDA receptors*. Journal of Neuroscience, 17, 8129–8136, 1997.

MathWorks, **May 2014a**. MathWorks. *qqplot*, 2014. URL http://www.mathworks.com.au/help/stats/qqplot.html.

MathWorks, **May 2014b**. MathWorks. *signrank*, 2014. URL http://www.mathworks.com.au/help/stats/signrank.html.

McMahon and Koltzenburg, **2006**. Stephen McMahon and Martin Koltzenburg. *Wall and Melzack's Textbook of Pain*. Elsvier Churchill Livingstone, 5th edition, 2006.

Miles and Clarkson, **1994**. MP Miles and PM Clarkson. *Exercise-induced muscle pain, soreness, and cramps*. Journal of Sports Medicine and Physical Fitness, 34, 203–216, 1994.

Morch, Andersen, Quevedo, Arendt-Nielsen, and Coghill, **2010**. Carsten Dahl Morch, Ole K. Andersen, Alexandre S. Quevedo, Lars Arendt-Nielsen, and Robert C. Coghill. *Exteroceptive aspects of nociception: Insights from graphesthesia and two-point discrimination*. Pain, 151, 42–52, 2010.

Nie, Arendt-Nielsen, Andersen, and Graven-Nielsen, **2005**. Hongling Nie, Lars Arendt-Nielsen, Helle Andersen, and Thomas Graven-Nielsen. *Temporal Summation of Pain Evoked by Mechanical Stimulation in Deep and Superficial Tissue*. The Journal of Pain, 6, 348–355, 2005.

Nielsen and Arendt-Nielsen, **1998**. J. Nielsen and L. Arendt-Nielsen. *The importance of stimulus configuration for temporal summation of first and second pain to repeated heat stimuli.* Eur J Pain, 2, 329–341, 1998.

Niesters, Hoitsma, Sarton, Aarts, and Dahan, **2011**. Marieke Niesters, Elske Hoitsma, Elise Sarton, Leon Aarts, and Albert Dahan. *Offset Analgesia in Neuropathic Pain Patients and Effect of Treatment with Morphine and Ketamine.* Anesthesiology, 5, 1063–1071, 2011.

Nilsson, Piasco, Nissen, Graversen, Gazerani, Lucas, Dahan, Drewes, and Brock, **2013**. M. Nilsson, A. Piasco, T.D. Nissen, C. Graversen, P. Gazerani, M.-F. Lucas, A. Dahan, A.M. Drewes, and C. Brock. *Reproducibility of psychophysics and electroencephalography during offset analgesia.* European journal of pain, pages 1–11, 2013.

Ken Nosaka. Muscle soreness and damage and the repeated-bout effect. In *Skeletal muscle damage and repair*, pages 59–76. Human Kinetics, 2008.

Oyeka and Ebuh, **2012**. Ikewelugo Cyprian Anaene Oyeka and Godday Uwawunkonye Ebuh. *Modified Wilcoxon Signed-Rank Test.* Open Journal of Statistics, 2, 172–176, 2012.

Polianskis, Graven-Nielsen, and Arendt-Nielsen, **2001**. Romanas Polianskis, Thomas Graven-Nielsen, and Lars Arendt-Nielsen. *Computer-controlled pneumatic pressure algometry - a new technique for quantitative sensory testing.* European Journal of Pain, 5, 267–277, 2001.

Polianskis, Graven-Nielsen, and Arendt-Nielsen, **2002**. Romanas Polianskis, Thomas Graven-Nielsen, and Lars Arendt-Nielsen. *Pressure-pain function in desensitized and hypersensitized muscle and skin assessed by cuff algometry.* The Journal of Pain, 3, 28–37, 2002.

Price, Rafiia, Watkinsa, and Buckinghama, **1984**. D.D. Price, A. Rafiia, L.R. Watkinsa, and B. Buckinghama. *A psychophysical analysis of acupuncture analgesia.* Pain, 19, 27–42, 1984.

Price, Gruen, Miller, Rafii, and Price, **1985**. D.D. Price, A. Von der Gruen, J. Miller, A. Rafii, and C. Price. *A psychophysical analysis of morphine analgesia.* Pain, 22, 261–269, 1985.

Price, Mao, Frenk, and Mayer, **1994**. DD. Price, J. Mao, H. Frenk, and DJ. Mayer. *The N-methyl-D-aspartate receptor antagonist dextromethorphan selectively reduces temporal summation of second pain in man.* Pain, 59, 165–174, 1994.

Purves, Augustine, Fitzpatrick, Hall, LaMantia, and White, **2012**. Dale Purves, George J Augustine, David Fitzpatrick, William C. Hall, Anthony-Samuel LaMantia, and Leonard E. White. *Neuroscience.* Sunderland, fifth edition edition, 2012.

Razali and Wah, **2011**. Nornadiah Mohd Razali and Yap Bee Wah. *Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling tests.* Journal of Statistical Modeling and Analytics, 2, 21–33, 2011.

Ren, **1994**. K Ren. *Wind-up and the NMDA receptor: from animal studies to humans.* Pain, 59, 157–158, 1994.

Reynolds, **1969**. D. V. Reynolds. *Surgery in the rat during electrical analgesia induced by focal brain stimulation.* Science, 164, 444–445, 1969.

Robinson, Wise, Gagnon, Fillingim, and Price, **2004**. Michael E. Robinson, Emily A. Wise, Christine Gagnon, Roger B. Fillingim, and Donald D. Price. *Influences of gender role and anxiety on sex differences in temporal summation of pain.* The Journal of Pain, 5, 77–82, 2004.

Sandkuhler and Gebhart, **1984**. J. Sandkuhler and G. F. Gebhart. *Relative contributions of the nucleus raphe magnus and adjacent medullary reticular formation to the inhibition by stimulation in the periaqueductal gray of a spinal nociceptive reflex in the pentobarbital-anesthetized rat.* Brain Research, 305, 77–87, 1984.

Sarlani, Grace, Reynolds, and Greenspan, **2004**. Eleni Sarlani, Edward G. Grace, Mark A. Reynolds, and Joel D. Greenspan. *Sex differences in temporal summation of pain and aftersensations following repetitive noxious mechanical stimulation.* Pain, 109, 115–123, 2004.

Schmelz, Schmidt, Bickel, Handwerker, and Torebjork, **1997**. Martin Schmelz, Roland Schmidt, Andreas Bickel, Hermann O. Handwerker, and H. Erik Torebjork. *Specific C-Receptors for Itch in Human Skin.* The Journal of Neuroscience, 17, 8003–8008, 1997.

Schmidt, Schmelz, Forster, Ringkamp, Torebjork, and Handwerker, **1995**. Roland Schmidt, Martin Schmelz, Clemens Forster, Matthias Ringkamp, Erik Torebjork, and Hermann Handwerker. *Novel classes of responsive and unresponsive C nociceptors in human skin.* The Journal of Neuroscience, 15, 333–341, 1995.

Siegel and Sapru, **2011**. Allan Siegel and Hreday N Sapru. *Essential Neuroscience.* The Point, 2nd edition, 2011.

Silbernagl and Despopoulos, **2009**. Stefan Silbernagl and Agamemnon Despopoulos. *Color Atlas og Physiology.* Thieme, 6th edition, 2009.

Sindhu and Shechtman, **2011**. Bhagwant S. Sindhu and Orit Shechtman. *Validity, Reliability, and Responsiveness of a Digital Version of the Visual Analog Scale.* Journal of hand therapy, 24, 356–364, 2011.

Skou, Graven-Nielsen, Lengsoe, Simonsen, Laursen, and Arendt-Nielsen, **2012**. Soren T. Skou, Thomas Graven-Nielsen, Lasse Lengsoe, Ole Simonsen, Mogens B. Laursen, and Lars Arendt-Nielsen. *Relating clinical measures of pain with experimentally assessed pain mechanisms in patients with knee osteoarthritis.* Scandinavian Journal of Pain, 4, 111–117, 2012.

Skou, Graven-Nielsen, Lengsoe, Simonsen, Laursen, and Arendt-Nielsen, **2013a**. Soren T. Skou, Thomas Graven-Nielsen, Lasse Lengsoe, Ole Simonsen, Mogens B. Laursen, and Lars Arendt-Nielsen. *Relating clinical measures of pain with experimentally assessed pain mechanisms in patients with knee osteoarthritis.* Scandinavian Journal of Pain, 4, 111–117, 2013.

Skou, Graven-Nielsen, Rasmussen, Simonsen, Laursen, and Arendt-Nielsen, **2013b**. Soren Thorgaard Skou, Thomas Graven-Nielsen, Sten Rasmussen, Ole H. Simonsen, Mogens B. Laursen, and Lars Arendt-Nielsen. *Widespread sensitization in patients with chronic pain after revision total knee arthroplasty.* Pain, 154, 1588–1594, 2013.

Smith, **2009**. Howard S. Smith. *Opioid Metabolism.* Mayo Clin Pro, 84, 613–624, 2009.

Staud, Cannon, Mauderli, Robinson, Price, and Vierck, **2003**. R Staud, RC Cannon, AP Mauderli, ME Robinson, DD Price, and CJ Jr Vierck. *Temporal summation of pain from mechanical stimulation of muscle tissue in normal controls and subjects with fibromyalgia syndrom.* Pain, 102, 87–95, 2003.

Staud, Koo1, Robinson, , and Price, **2007**. Roland Staud, Euna Koo1, Michael E. Robinson, , and Donald D. Price. *Spatial Summation of Mechanically Evoked Muscle Pain and Painful Aftersensations in Normal Subjects and Fibromyalgia Patients.* Pain, 130, 177–187, 2007.

Streiner and Norman, **2011**. David L. Streiner and Geoffrey R. Norman. *Correction for multiple testing. Is there a resolution?* Chest, 140, 16–18, 2011.

Karakaya, Karakaya, Ergun, Elmalõ, and Fõrat, **2014**. Ilkim ,õtak Karakaya, Mehmet GŸrhan Karakaya, Esra Ergun, Sedanur Elmalõ, and Tuzun Fõrat. *Effects of different frequencies of conventional transcutaneous electrical nerve stimulation on pressure pain threshold and tolerance.* Journal of Back and Musculoskeletal Rehabilitation, 27, 197–201, 2014.

Trojan, Stolle, Kleinbohl, Morch, Arendt-Nielsen, and Holzl, **2006**. Jorg Trojan, Annette M. Stolle, Dieter Kleinbohl, Carsten D. Morch, Lars Arendt-Nielsen, and Rupert Holzl. *The saltation illusion demonstrates integrative processing of spatiotemporal information in thermoceptive and nociceptive networks.* Exp Brain Res, 170, 88–96, 2006.

Tuncali, Karci, Tuncali, Mavioglu, Ozkan, Bacakoglu, Baydur, Ekin, and Elar, **2006**. B. Tuncali, A Karci, B.E. Tuncali, O Mavioglu, M Ozkan, A.K. Bacakoglu, H. Baydur, A. Ekin, and Z. Elar. *A new method for estimating arterial occlusion pressure in optimizing pneumatic tourniquet inflation pressure.* Anesthesia and Analgesia, 102, 1752–1757, 2006.

Vanegasa and Schaible, **2004**. Horacio Vanegasa and Hans-Georg Schaible. *Descending control of persistent pain: Inhibitory or facilitatory?* Brain Research Reviews, 46, 295–309, 2004.

Weidner, Schmelz, Schmidt, Hansson, Handwerker, and Torebjork, **1997**. C. Weidner, M. Schmelz, R. Schmidt, B. Hansson, H. O. Handwerker, and H. E. Torebjork. *Specific C-Receptors for Itch in Human Skin.* The Journal of Neuroscience, 17, 8003–8008, 1997.

Yarnitsky and Pud, **2004**. D Yarnitsky and D. Pud. *Quantitative sensory testing.* Muscle & nerve, pages 305–332, 2004.

Yarnitsky, Arendt-Nielsen, Bouhassira, Edwards, Fillingim, Granot, Hansson, Lautenbacher, Marchand, and Wilder-Smith, **2010**. D Yarnitsky, L Arendt-Nielsen, D Bouhassira, RR Edwards, RB Fillingim, M Granot, P Hansson, S Lautenbacher, S Marchand, and O Wilder-Smith. *Recommendations on terminology and practice of psychophysical DNIC testing.* Eur J Pain, 14, 339, 2010.

Yarnitsky, **2010**. David Yarnitsky. *Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states.* Current opinion in anaesthesiology, 23, 611–615, 2010.

Yelle, Rogers, and Coghill, **2008a**. Marc D. Yelle, June M. Rogers, and Robert C. Coghill. *Offset analgesia: A temporal contrast mechanism for nociceptive information.* Pain, 134, 174–186, 2008.

Yelle, Rogers, and Coghill, **2008b**. Marc D. Yelle, June M. Rogers, and Robert C. Coghill. *Offset analgesia: A temporal contrast mechanism for nociceptive information.* Pain, 134, 174–186, 2008.

Yelle, Oshiro, Kraft, and Coghill, **2009**. Marc D. Yelle, Yoshitetsu Oshiro, Robert A. Kraft, and Robert C. Coghill. *Temporal filtering of nociceptive information by dynamic activation of endogenous pain modulatory systems.* The Journal of Neuroscience, 29, 10264–10271, 2009.

Yu and Thornell, **2002**. JG Yu and LE Thornell. *Eccentric contractions leading to DOMS do not cause loss of desmin nor fibre necrosis in human muscle.* Histochemistry and Cell Biology, 118, 29–34, 2002.

Zar, **2010**. Jerrold H. Zar. *Biostatistical analysis.* Pearson, 5th edition, 2010.