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Short report

Tapentadol and oxycodone reduce cingulate glutamate in healthy volunteers

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Short title: Tapentadol and oxycodone reduce cingulate glutamate

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Keywords: Magnetic resonance spectroscopy; Opioid; Glutamate; Oxycodone; Tapentadol

What is already known about this subject:

- Limited knowledge exists about the effects of tapentadol on the central nervous system
- Magnetic resonance spectroscopy can be a valuable tool to objectively evaluate the effects of analgesics
- Glutamate is a neurotransmitter, which has been shown to be modulated during analgesic treatments

What this study adds:

- Decreased levels of glutamate/creatine were demonstrated in the anterior cingulate cortex after treatment with both tapentadol and oxycodone
- This may reflect that both treatments modulate the glutamatergic system at the supraspinal level

Abstract:

Tapentadol and oxycodone are commonly used analgesics. Preclinical studies have shown that oxycodone modulates brain metabolites related to opioid pathways, whereas tapentadol also affects noradrenergic activity. However, knowledge about the function of the medications in the human brain is limited. The aim was to investigate effects of tapentadol and oxycodone on brain glutamate, the most important neurotransmitter in pain processing. Magnetic resonance spectroscopy was obtained in 21 healthy subjects from the anterior cingulate cortex, prefrontal cortex and insula at baseline and after 14 days of treatment with either 50mg tapentadol, 10mg oxycodone (equipotent dose, both extended release) or placebo BID in a randomized double-blind cross-over study. Compared to baseline, decreased glutamate/creatine levels were identified in anterior cingulate cortex after tapentadol (1.26±0.14 vs. 1.35±0.18, p=0.04) and oxycodone (1.26±0.10 vs. 1.35±0.12, p=0.05) treatments, both with 7% reduction. This indicates that both analgesics modulate the glutamatergic system at the supraspinal level in humans.

Introduction

Severe pain is often treated with classical opioids, but treatment is far from optimal as the individual analgesic efficacy and frequently reported side effects challenges the benefits of the treatments. Classical opioids have effects on the peripheral, spinal and supraspinal levels. The binding to opioid receptors blocks the neurotransmitter release of glutamate and other neurotransmitters to obtain pain relief (1). Other drug classes such as serotonin-norepinephrine reuptake inhibitors (SNRIs) and noradrenaline reuptake inhibitors (NRI) also exert analgesic effects, but through other mechanisms mainly in the brainstem. Tapentadol has two mechanisms as it exerts its analgesic properties by combining μ-opioid receptor agonistic affinity with noradrenaline reuptake inhibition (2). The noradrenergic activity may provide a genuine opioid-sparing effect, maintaining analgesic efficacy despite reduced μ-opioid receptor affinity and at the same time reducing typical opioid-related side effects (2,3). To obtain more knowledge into the central mechanisms of tapentadol, it is relevant to investigate the effects in the brain as compared to pure opioids. Preclinical studies have shown that pure opioids modulate brain metabolites related to opioid pathways (4,5), whereas tapentadol also affect noradrenergic activity (2). Previously magnetic resonance spectroscopy (MRS) has been used to detect brain metabolites following treatment with analgesics, and can be a tool to objectively evaluate the central pain processing and effects of analgesics (6,7). Recently, we have examined the effects of oxycodone (opioid) and venlafaxine (SNRI) treatments on brain metabolites (8). Only, the glutamate/creatine (glu/cre) ratio decreased after 5 days of oxycodone treatment in brain regions with high opioid-receptor density (8). We hypothesized that the levels of glutamate would also decrease after treatment with tapentadol. Thus, the aim of the study was to investigate the effects of

tapentadol, oxycodone and placebo on brain glutamate in healthy subjects using MRS. Thus, we investigated the glu/cre levels in the anterior cingulate cortex (ACC), the prefrontal cortex (PFC) and insula, which are brain regions known to be important in pain processing and rich in opioid receptors (9–11). Furthermore, associations between the glu/cre ratio and experimental induced pain scores were explored.

Methods

The study was a randomized, double-blinded, placebo-controlled, cross-over study in 21 healthy subjects investigating the effects of 14 days of treatment with tapentadol, oxycodone and placebo on brain glutamate. Treatment periods of 14 days were chosen to ensure adequate treatment as previous studies indicate that tapentadol's NRI modulation is slowly increasing and reach its maximal effect after two weeks (12). The study was a part of a larger study with the primary endpoint to investigate the analgesic mechanisms of tapentadol on different levels of the nervous system (EUDRACT ref 2017-000141-52). Thus, the sample size was calculated based on the primary endpoint as described in another publication (13). The sample size was considered adequate to investigate glu/cre as an explorative outcome and is also in line with sample sizes used in similar investigations (8,14). Magnetic resonance imaging (MRI) scans including MRS were performed between 8 to 10 in the morning on day 1 before treatment start (baseline measurements) and on day 14 (after each treatment). Each subject underwent all three treatment arms (six MRI scans in total per subject). All subjects were informed thoroughly about the study and gave written, informed consent before participating. The inclusion criteria were healthy males, age between 20 and 45 years, Scandinavian descent, and ability to read and understand

Danish. Study approvals were obtained from The North Denmark Region Committee on Health Research Ethics (N-20170009) and the Danish Medicines Agency (2017041794). The Good Clinical Practice unit at Aalborg and Aarhus University Hospitals, Denmark monitored the study.

Medication

Treatments were administered during a period of 14 days for each treatment arm in a randomized order with minimum one-week wash-out between treatments. Tapentadol (Palexia®, 50 mg, extended release), oxycodone (OxyContin®, 10 mg, extended release) or placebo tablets were administered orally on day 1 (after the first scan, meaning that the first scan was pre-treatment) and day 14 (just before the scan, meaning that the last scan was taken still under treatment) once and on day 2 to 13 twice a day (morning and evening). The Hospital Pharmacy Aarhus, Aarhus University Hospital, Aarhus, Denmark handled the tablets, which were encapsulated in DPcaps® (red color, size AA, 13.07-14.44 x 9.39mm, Capsugel®) to look identical.

Data acquisition and post processing

The MRI scans including MRS were performed on a 3T GE scanner (GE Signa HDxt, General Electric, Milwaukee, WI, USA) using a standard eight-channel head coil. Foam pads were used to minimize head movements. Three single voxel PRESS (Point RESolved Spectroscopy) ^{1}H-MRS measurements were acquired (TR/TE = 2000/30 ms) with a scan time of 5 minutes and total number of scans of 128 for each voxel. These were the ACC (20x20x20 mm), the PFC (15x15x20 mm) and the right insula (15x20x50 mm) as illustrated in figure 1. The ACC and PFC voxels were positioned on a high resolution sagittal T2-weighted fast spin echo sequence. The

ACC voxel was placed in the midline of the pregenual ACC with the inferior border along the anterior-posterior commissure line and the PFC voxel was placed in the midline superior to the ACC voxel (no overlap). The insula voxel was placed in insula on a high-resolution axial T1-weigthed structural scan. Post processing of the measurements of were performed in LCModel (Version 6.3) (15). Data were fitted in the chemical shift range 0.1 ppm to 4.0 ppm. Metabolites (glu/cre, N-acetylaspartate/cre and myo-Inositol/cre) with sufficient quality (Cramér-Rao bounds ≤15%) were included for further investigations. Metabolite estimates are highly dependent on e.g., the MRS protocol, scanner and size and placement of the voxel of interest (16–19). Therefore, based on data from our previous study using the same protocol and scanner (baseline data) (8), only glu/cre measurements inside the range of the mean±2 x standard deviation (SD) were considered and data points outside the adjacent lines of the corresponding boxplots (outliers) were excluded as these were considered to be unreliable values due to e.g. noise and head movements. N-acetylaspartate/cre and myo-Inositol/cre ratios were provided as additional descriptive data to explore any systematic bias in metabolite changes.

Experimental induced pain

A cold pressor test was used to evoke experimental pain and to assess subjective pain perception (20). The participant immersed the hand in 2 ºC cold water (stirred by a pump from Grant, Fisher Scientific, Slangerup, Denmark) for 120 seconds and rated the related pain perception on a visual analogue scale (VAS) from 0-10 (0=no pain, 10=worst imaginable pain). The test was performed after the MRI session at baseline, day 4 and day 14.

Statistical analysis

Data were compared for the glu/cre ratio for each MRS voxel and for VAS ratings using a repeated measures mixed effects model with treatments and time as fixed effects (full factorial) and subject as a random effect. The linear mixed model was chosen as this estimation method is able to handle missing data without excluding subjects listwise. Associations between the glu/cre ratio during treatment and experimental induced pain scores were explored using Spearman's correlation for areas with significant changes in glu/cre, including data from all treatments. The statistical analyses were performed in STATA version 15 (StataCorp LP, College Station, TX, USA). Data are presented as mean±SD and $p \leq 0.05$ was considered significant.

Results

21 healthy subjects (age: 24.9±2.7 years) finalized the study. Data from three subjects were excluded for the ACC analysis and one subject´s data were excluded from the insula analysis as data qualities were poor. Additionally, unreliable values (as described in the Methods section) were not included. The mean data quality measurements and the numbers of included subjects for each area, time and treatment are summarized in Table 1.

Glu/cre findings before (baseline) and after all treatments for the three predefined areas are summarized in Table 1 and figure 2. Differences in glu/cre were only identified in the ACC (overall p=0.045 for ACC, p=0.478 for PFC, p=0.140 for insula) with decreased levels after treatment as compared to baseline for both the tapentadol (p=0.04) and oxycodone (p=0.05) treatments. No difference was seen for

placebo as compared to baseline (p=0.6). Overall, the metabolite reduction was 7% after both oxycodone and tapentadol treatment compared to baseline (placebo: 1% reduction). Additional metabolite levels (N-acetylaspartate/cre and myo-Inositol/cre) are provided in table 1. VAS ratings (overall p<0.01) decreased after treatment compared to baseline for both tapentadol (day 4, p=0.01 and day 14, p=0.09) and oxycodone (day 4, p=0.001 and day 14, p=0.01) treatments but not for placebo (day 4, p=0.7 and day 14, p=0.5), see Table 1. No association was demonstrated between the ACC glu/cre ratio and VAS ratings after treatments (r=-0.08, p=0.6).

Discussion

In this randomized, double-blinded, placebo-controlled, cross-over study, magnetic resonance spectroscopy was used to investigate metabolite levels after tapentadol and oxycodone treatments in the brain regions most relevant for pain processing. The glu/cre level decreased after 14 days of treatment for both active treatments in the anterior cingulate cortex potentially reflecting similar modulation of the glutamatergic system.

Glutamate is an excitatory neurotransmitter involved in neuronal activity (21) and is a key metabolite involved in pain transmission (22). In this present study decreased glu/cre levels were demonstrated in the ACC after both tapentadol and oxycodone treatments, which might indicate decreased neuronal activity. Such a decrease in the glu/cre level has previously been identified after 5 days of treatment with oxycodone (8). Furthermore, oxycodone has also been demonstrated to decrease functional coupling during resting state between limbic structures and supra limbic areas (23,24). Oxycodone is a strong opioid and a decrease in glu/cre levels could reflect the inhibitory effect of oxycodone on mu-receptors, as activation

of these receptors reduces transmitter release. Venlafaxine, which is a serotonin-norepinephrine reuptake inhibitor (SNRI) previously showed a trend towards lower glu/cre levels in the ACC, PFC and insula (8). Serotonin and noradrenaline are neurotransmitters released by the descending pathways of the central nervous system and are involved in modulation of the flow of noxious information (25). The descending noradrenergic pathway mainly facilitates pain inhibition. Tapentadol acts through two mechanisms, exerting its analgesic properties by combining μ-opioid receptor agonistic affinity with noradrenaline reuptake inhibition (2,3,25). This dual effect may act synergistical in terms of analgesic efficacy (2,3,25). Thus, the decreased ACC glu/cre levels could possibly be explained by both direct and/or indirect effect on the opioid receptors and by the top-down influence on descending inhibitory pathways. However, the different mechanisms cannot be distinguished in this study.

MRS is a method that has previously shown to be reproducible and reliable using the same scanner and protocol (26,27). However, the method is prone to e.g. head motion which will affect the data quality and signal to noise ratio (28). As this study was in healthy volunteers, outlier data were considered to be due to quality issues and were not included in the analysis. Even though missing data points lowered the sample size, previous studies investigated similar sample sizes (8,14). However, these results should still be validated in larger samples across multiple scanners and MRS protocols.

The glu/cre ratio is frequently investigated and easy to implement (29). However, using this ratio to report changes in glutamate levels, the creatine level is assumed to be constant, but it is possible that the creatine level can also be affected. Furthermore, glutamine is synthesized from glutamate and these metabolites are

closely connected in brain metabolism. These can be difficult to distinguish (30) but it seems that glutamate can be reliable measured at PRESS TE=30 as used in this present study (18).

In our study, ACC, PFC and insula were investigated as these areas are involved in pain processing and are rich in opioid receptors (31–34). The size of the voxel of interest would impact the signal to noise ratio and affects the interpretation of the glu/cre ratios. To support our results to be related to the drug effects, a control area with no or low density of opioid receptors could be investigated in future studies. We only found significantly decreased glu/cre levels in the ACC. ACC is a part of the limbic area where the affective component of pain is generated. Furthermore, previous studies have found that the ACC glutamate level was increased and associated with pain in chronic pain conditions (35–37). In this present study, no association was demonstrated between the glu/cre ratio and experimentally induced pain scores which was obtained after the MRI scan. Thus, the study showed decreased glu/cre after oxycodone and tapentadol treatments in the absence of pain. Glutamatergic neurotransmission might therefore be a key to modulate and potentially explain the pain relief from analgesics in chronic pain disorders. This could involve both decreased activation of higher structures involved in pain and in cognitive/affective processing and the top-down influence on descending inhibitory pathways. Finally, even though MRS could be useful as an objective measure of the treatment effect, the decrease in glu/cre could be related to several central effects of the treatments, including side-effects. From the same study, it was shown that oxycodone induced gastrointestinal side-effects associated with prolonged whole gut and colonic transit times (13,38). Tapentadol also increased the colonic volume, but transit times and symptom scores did not differ from placebo (13,38). Both efficacy

and side-effects of the treatments can be so obvious that blinding in placebo-controlled trials can be difficult to maintain after the first period in cross-over trials.

This present study was conducted in healthy subjects as mechanistic effects are more difficult to evaluate in clinical settings due to confounding effects from comorbidity and comedication (39). Thus, the potential treatment effects can be difficult to translate to clinical pain and the effects with higher doses might be different (40).

In conclusion, the glu/cre level decreased after 14 days of treatment with tapentadol and oxycodone in the anterior cingulate cortex. This could potentially reflect that both treatments modulate the glutamatergic system at the supraspinal level in humans.

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Data availability:

The data that support the findings of this study are available from the corresponding author upon request.

Nomenclature of ligands:

Key ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (41).

Authorship statement:

Study design and original idea: TMH, JBF, AMD; Data collection and analysis: TMH, JBF, EBM, AMD; Drafting the manuscript: TMH, JBF; All authors contributed to the literature search preparation of the manuscript and critical revisions therein regarding important intellectual content. All authors have approved the final manuscript.

Disclosure:

The authors declare no conflicts of interest.

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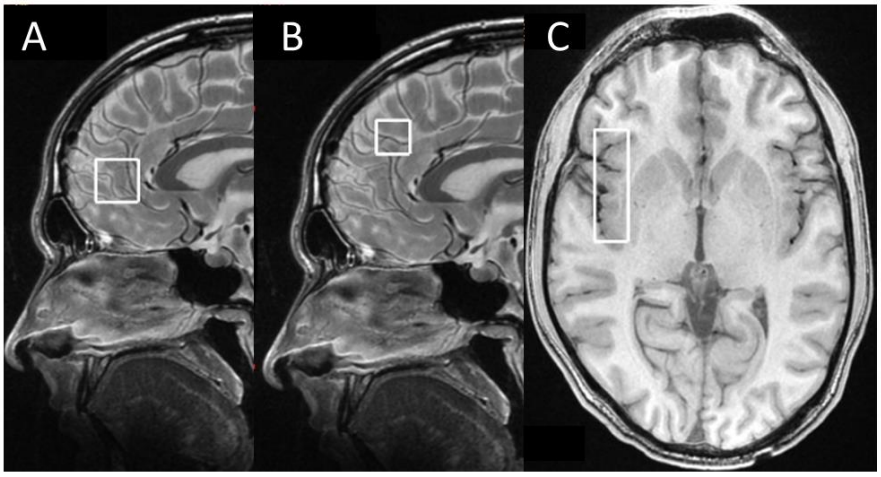

Figure 1: An example of the positions of the voxels of interests in the midline pregenual anterior cingulate cortex (A), midline prefrontal cortex (B) superior to the voxel of interest in the anterior cingulate cortex and insula (C).

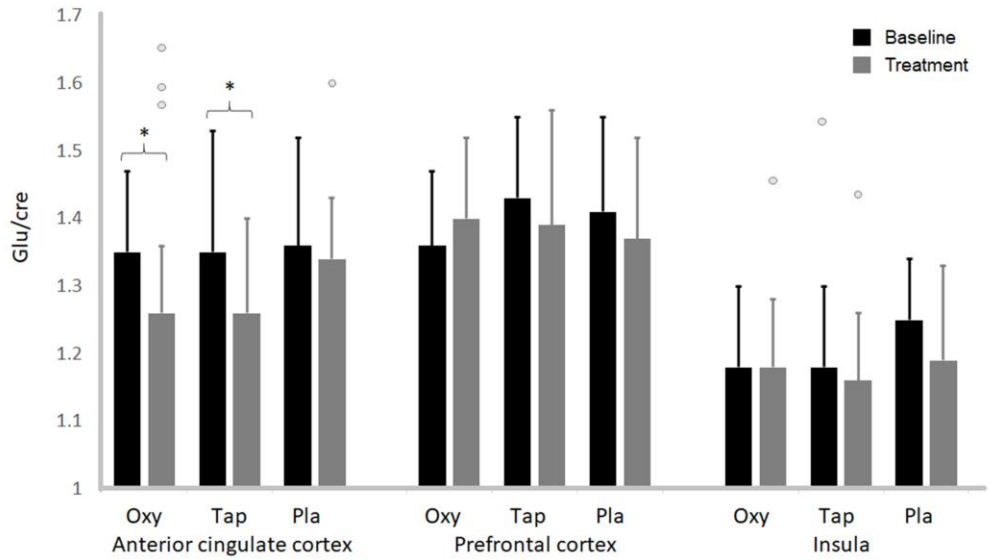

Figure 2: The mean (standard deviation) glutamate/creatine (glu/cre) levels at baseline (black) and after 14 days treatment (gray) for the three treatments, oxycodone (Oxy), tapentadol (Tap) and placebo (Pla) estimated in the anterior cingulate cortex, the prefrontal cortex and the right insula. * indicates significant changes between baseline and treatment levels ($p \leq 0.05$). Outlier data (dots) are shown but not included in the illustrated mean (standard deviation) and statistics.

Table 1: Magnetic resonance spectroscopy measurements, data quality measurements (glu/cre measurements highlighted in boxes) and VAS ratings of pain perception during an experimental cold pressor test.

	Oxycodone arm		Tapentadol arm		Placebo arm	
	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment
Anterior cingulate cortex	n=17	n=15	n=17	n=18	n=17	n=15
Glu/cre	1.35±0.12	1.26±0.10	1.35±0.18	1.26±0.14	1.36±0.16	1.34±0.09
Glu CRLB (%)	7.5±1.3	8.8±1.8	8.1±2.3	8.5±2.4	8.2±2.1	7.7±1.2
Cre CRLB (%)	3.4±0.7	3.5±0.8	3.6±0.9	3.4±0.8	3.7±0.7	3.4±0.6
NAA/cre	1.11±0.10	1.12±0.10	1.13±0.11	1.10±0.11	1.14±0.09	1.12±0.12
NAA CRLB (%)	3.9±1.1	4.4±1.2	4.1±1.6	4.2±2.0	4.2±1.1	4.1±1.1
mI/cre	0.77±0.08	0.77±0.11	0.81±0.10	0.71±0.13	0.74±0.15	0.77±0.11
mI CRLB (%)	5.8±1.2	6.4±1.8	6.2±1.7	6.9±2.4	6.8±2.3	6.1±1.1
Signal-to-noise ratio	15.9±5.7	14.6±5.3	15.7±5.6	15.0±5.4	14.0±5.4	15.1±4.6
Full width at half maximum	0.044±0.015	0.043±0.012	0.048±0.011	0.046±0.012	0.049±0.013	0.048±0.019
Prefrontal cortex	n=20	n=20	n=19	n=21	n=21	n=20
Glu/cre	1.36±0.11	1.40±0.12	1.43±0.12	1.39±0.17	1.41±0.14	1.37±0.15
Glu CRLB (%)	7.8±1.2	7.6±0.9	7.5±1.3	7.9±1.1	7.8±0.9	7.8±1.6
Cre CRLB (%)	3.3±0.4	3.3±0.5	3.4±0.6	3.3±0.6	3.3±0.5	3.4±0.6
NAA/cre	1.34±0.09	1.35±0.13	1.36±0.13	1.35±0.07	1.34±0.08	1.37±0.13
NAA CRLB (%)	3.3±0.5	3.4±0.5	3.3±0.5	3.4±0.6	3.4±0.5	3.2±0.4
mI/cre	0.74±0.09	0.71±0.08	0.74±0.11	0.74±0.07	0.76±0.06	0.71±0.08
mI CRLB (%)	6.1±1.0	6.6±1.1	6.3±1.3	6.3±0.8	6.1±0.9	6.4±1.8
Signal-to-noise ratio	16.8±2.3	17.0±3.3	16.9±1.9	17.1±2.4	17.1±2.2	16.9±2.0
Full width at half maximum	0.035±0.006	0.033±0.005	0.036±0.006	0.035±0.009	0.035±0.006	0.036±0.007
Insula	n=20	n=19	n=19	n=19	n=20	n=20
Glu/cre	1.18±0.12	1.18±0.10	1.18±0.12	1.16±0.10	1.25±0.09	1.19±0.14
Glu CRLB (%)	6.3±0.9	6.2±0.8	5.9±0.8	6.1±0.9	5.7±0.7	6.1±1.0
Cre CRLB (%)	2.3±0.4	2.2±0.4	2.1±0.2	2.0±0.0	2.1±0.3	2.2±0.4
NAA/cre	1.28±0.13	1.28±0.10	1.28±0.08	1.27±0.07	1.27±0.08	1.26±0.09
NAA CRLB (%)	2.3±0.4	2.6±0.5	2.3±0.5	2.5±0.5	2.3±0.5	2.5±0.5
mI/cre	0.72±0.11	0.69±0.11	0.74±0.15	0.71±0.09	0.72±0.06	0.72±0.11
mI CRLB (%)	4.4±0.5	4.8±1.5	4.3±0.7	4.4±0.5	4.2±0.7	4.3±0.9
Signal-to-noise ratio	35.3±4.2	34.2±6.5	36.6±3.2	38.4±2.6	36.4±3.6	34.8±4.9
Full width at half maximum	0.045±0.009	0.048±0.013	0.045±0.006	0.043±0.004	0.044±0.009	0.048±0.008
Pain perception	n=21	n=21	n=21	n=21	n=21	n=21
VAS	6.2±2.0	5.5±1.8 (day 4) 5.6±1.9 (day 14)	6.3±1.8	5.7±1.8 (day 4) 5.9±1.8 (day 14)	6.0±1.8	5.9±1.6 (day 4) 5.9±1.8 (day 14)

Magnetic resonance spectroscopy measurements at baseline and treatment (day 14) are presented as mean ± standard derivation for each voxel of interest. *The glu/cre measurements were of main interests and highlighted in boxes.* The following data were excluded based on glu/cre measurements: for the anterior cingulate cortex, 3 subjects were excluded due to poor data quality in general. Out of the remaining subjects, 9 values were excluded as 3 values had Cramér-Rao lower bounds > 15%, 2 values were below 1.01 and 4 values were outliers. For the prefrontal cortex, no subjects were excluded across all treatments and time, but 5 values were excluded as 4 values were below 1.05 and one value was higher than 1.95. For the insula, one subject was excluded based on poor data quality in general and 3 values were outliers and excluded. Outliers are illustrated in Figure 2 and not included in the statistics. VAS ratings of pain perception during the experimental cold pressor test were presented as mean ± standard derivation. Glu: glutamate; Cre: creatine, NAA; n-acetylaspartate; mI; myo-Inositol; CRLB: Cramér-Rao lower bounds; n=number of subjects.