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N-20160054

EudraCT Number: 2016-002623-29

The Effect of Morphine on the Human Central Nervous System

Summary of the Project Outcomes

The project N-20160054 has been successfully completed as expected and all the data has been analyzed. We observed that morphine induced significant changes on a number of central parameters and majority of these was reversed by naloxone. This reversal is especially interesting because now we know which central changes are opioid specific. Based on this extensive study, we could conclude that the most sensitive assessments of opioid analgesia are done via nociceptive withdrawal reflexes and EEG responses to tonic pain. A manuscript reporting all the outcomes is currently under review in the Journal of Neuroscience.

This is the abstract of the article submitted to the journal:

Moderate to severe pain is often treated with opioids, but central mechanisms underlying opioid analgesia are poorly understood. Findings thus far have been contradictory and none could infer opioid specific effects. This study aimed to find opioid specific effects on central processing and subjective perception of external stimuli. Twenty healthy male volunteers were included in this placebo-controlled, randomized, two-way, cross-over, double-blinded study. Three sets of assessments were done on each of the two visits: 1) baseline, 2) during continuous morphine or placebo infusion and 3) during simultaneous morphine + naloxone or placebo infusion. Quantitative sensory testing (QST), spinal nociceptive withdrawal reflexes (NWR), spinal electroencephalography (EEG), cortical EEG responses to external stimuli and resting EEG were measured and analyzed. Longer lasting (cold-pressor test, tetanic electrical stimulation), deeper structure (bone pressure) and strong nociceptive (NWR) stimulations were the most sensitive QST measures of morphine analgesia. In line with this, the principal opioid specific central changes were seen in NWRs, EEG responses to NWRs and cold-pressor EEG. The magnitude of NWRs together with amplitudes and insular source strengths of the corresponding EEG responses were attenuated. The decreases in EEG activity were correlated to unpleasantness scores. Brain activity during cold-pressor test in slow EEG (1-4Hz) was decreased, whereas in faster EEG (8-12Hz) it was increased. These changes were related to pain scores. This study gives evidence of opioid specific effects on subjective perception of external stimuli and central responses. It also provides indication of the most sensitive methods to investigate central effects of opioids.

On the following pages, you will find a more detailed summary of the study design and results in figures and tables.

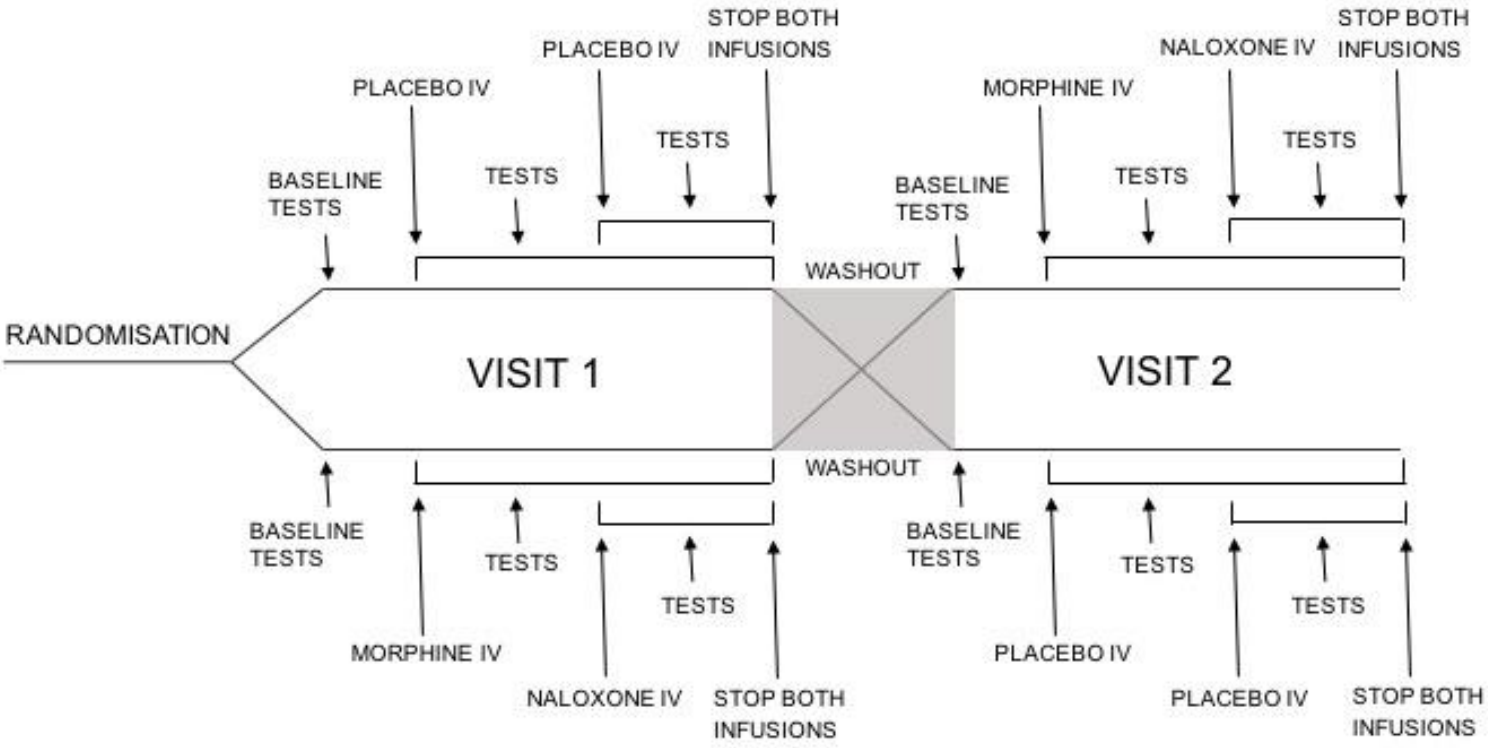

Figure 1. Study design. Tests are described more specifically in Figure 2.

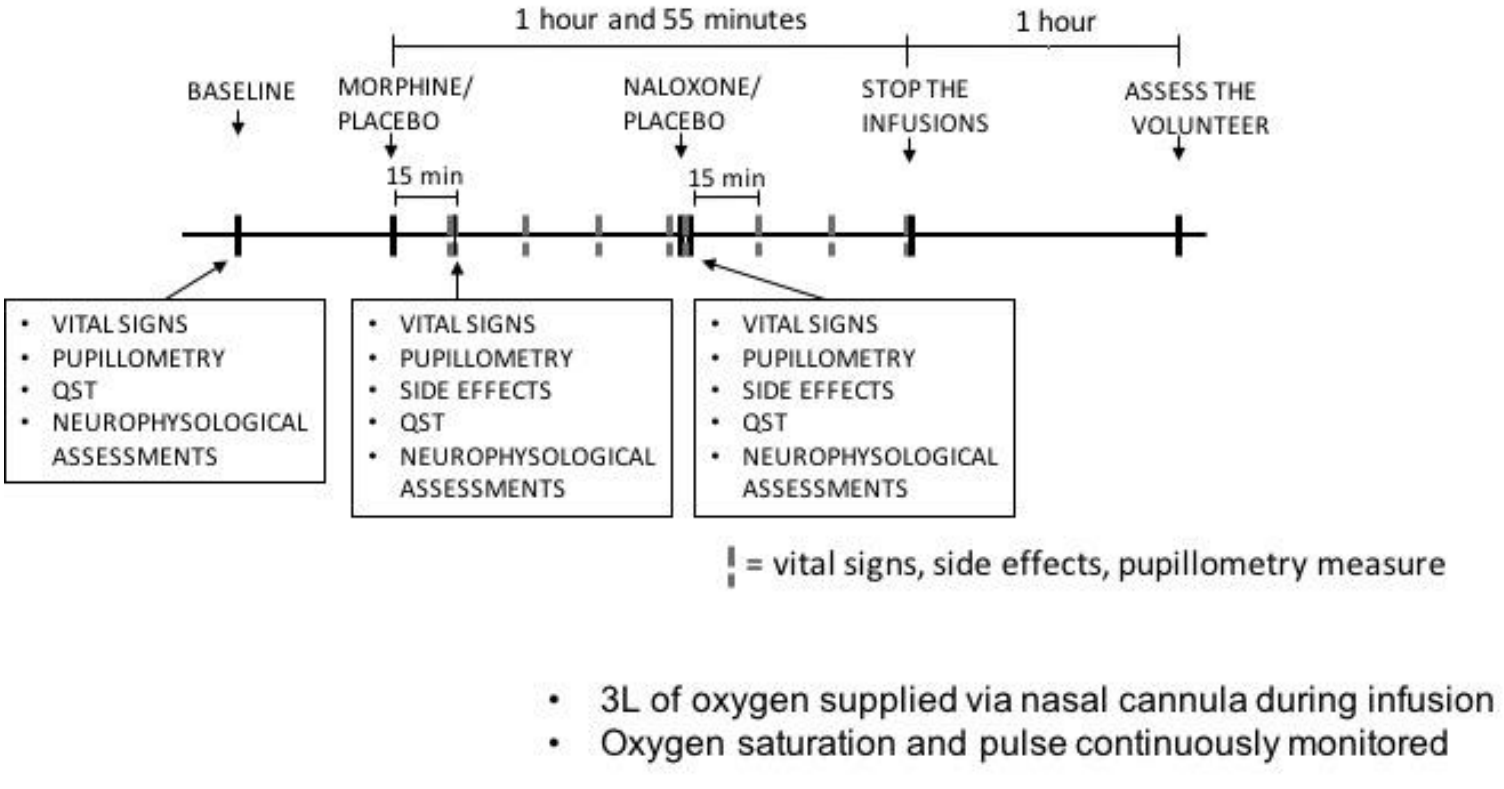

Figure 2. Experimental timeline for each visit. QST=quantitative sensory testing. QST measures were: nociceptive withdrawal reflex threshold, heat stimulation, offset analgesia, bone pressure, tetanic electrical stimulation, cold-pressor (hand in 2 degree water for 2 minutes) and conditioned pain stimulation. Neurophysiological assessments were: resting cortical EEG, cortical EEG

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response to NWR stimulations, EMG responses to NWR stimulations, EEG (both spinal and cortical) to median nerve stimulations, EEG responses to cold-pressor pain (tonic pain).

	PLACEBO				PLACEBO + PLACEBO				MORPHINE				MORPHINE + NALOXONE			
Time after continuous infusion start (min) →	15	30	45	60	65	80	95	110	15	30	45	60	65	80	95	110
Nausea (N)	0	0	0	0	0	0	0	0	4	2	4	3	5	1	0	0
Vomitting (N)	0	0	0	0	0	0	0	0	1	1	2	1	1	0	0	0
Headache (N)	0	2	1	0	0	0	0	0	1	0	2	1	3	2	2	0
Dizziness (N)	0	1	0	0	0	0	1	0	***9*****	***8*****	***8*****	***6*****	***5****	1	2	1
Sedation (N)	0	1	3	0	2	2	3	0	***11*****	***14******	***16******	***12******	5	***6****	***6****	***6****
Dry mouth (N)	1	2	1	1	1	1	2	2	***13*****	***13*****	***13*****	***9****	6	4	3	2
Itching (N)	0	0	0	0	0	0	0	0	3	3	4	3	4	0	0	0
Rapid HR (N)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Breathing pr. (N)	0	0	0	0	0	0	0	0	1	0	2	2	0	0	0	0
Sweating (N)	2	3	2	1	0	2	1	1	4	5	4	4	3	3	2	1
Stomach pain (N)	0	0	0	0	0	0	0	0	2	2	1	0	1	1	0	1
Gen. Discomf.(N)	0	0	0	0	0	0	0	0	5	4	5	4	6	2	2	1
Other (N)	0	0	0	0	0	0	0	0	4	3	2	2	3	1	0	0
Pupil diameter change in mm (mean (se))	0 (.1)	-.1 (.1)	-.1 (.1)	0 (.1)	0 (.1)	-.2 (.1)	0 (.1)	.1 (.1)	***-2.2*** (.2)***	***-2.7*** (.2)***	***-2.7*** (.2)***	***-2.8*** (.2)***	-.4 (.2)	-.5 (.1)	-.4 (.1)	-.5 (.1)

Table 1. Side effects and pupillometry. The numbers for *side effects* represent the number of subjects who experienced them. The side effects which fall under the 'other' category are: Tension in the body, heavy eyes, lump in the stomach for morphine, and cold in the body, cold in the stomach, weird taste in the mouth for naloxone. The numbers for *pupil diameter change* represent the change from baseline pupil diameter on the same day, before drug infusion was started. The highlighted cells represent the values which showed statistically significant changes. PLACEBO = first placebo infusion; PLACEBO + PLACEBO = second placebo infusion together with the first one; MORPHINE = lone morphine infusion; MORPHINE + NALOXONE = naloxone infusion together with morphine. Abbreviations: HR= heart rate; pr.=problems; gen.=general. **P*<0.05, ***P*<0.01,****P*<0.001.

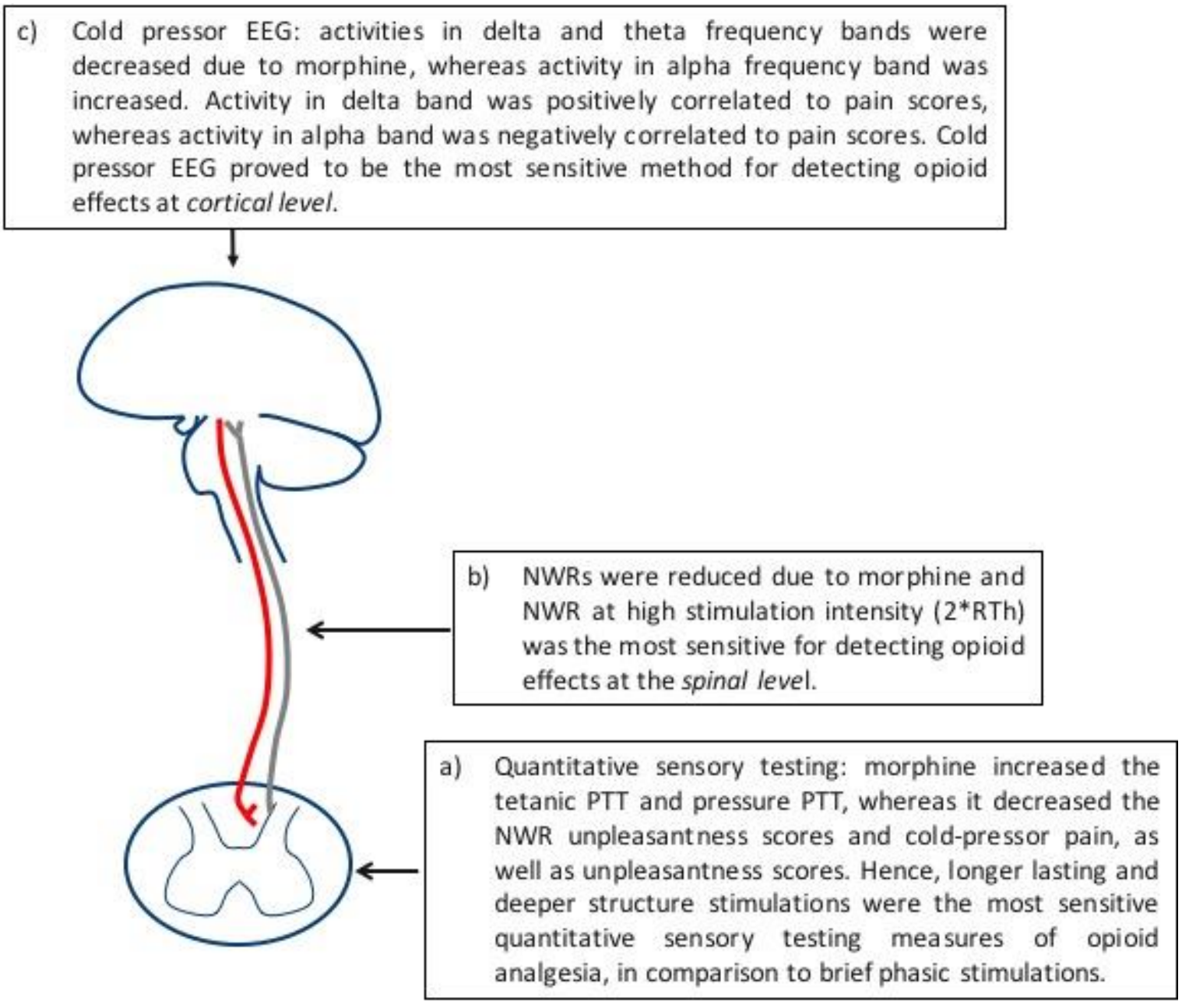

Figure 3. Simplified presentation of the main results.

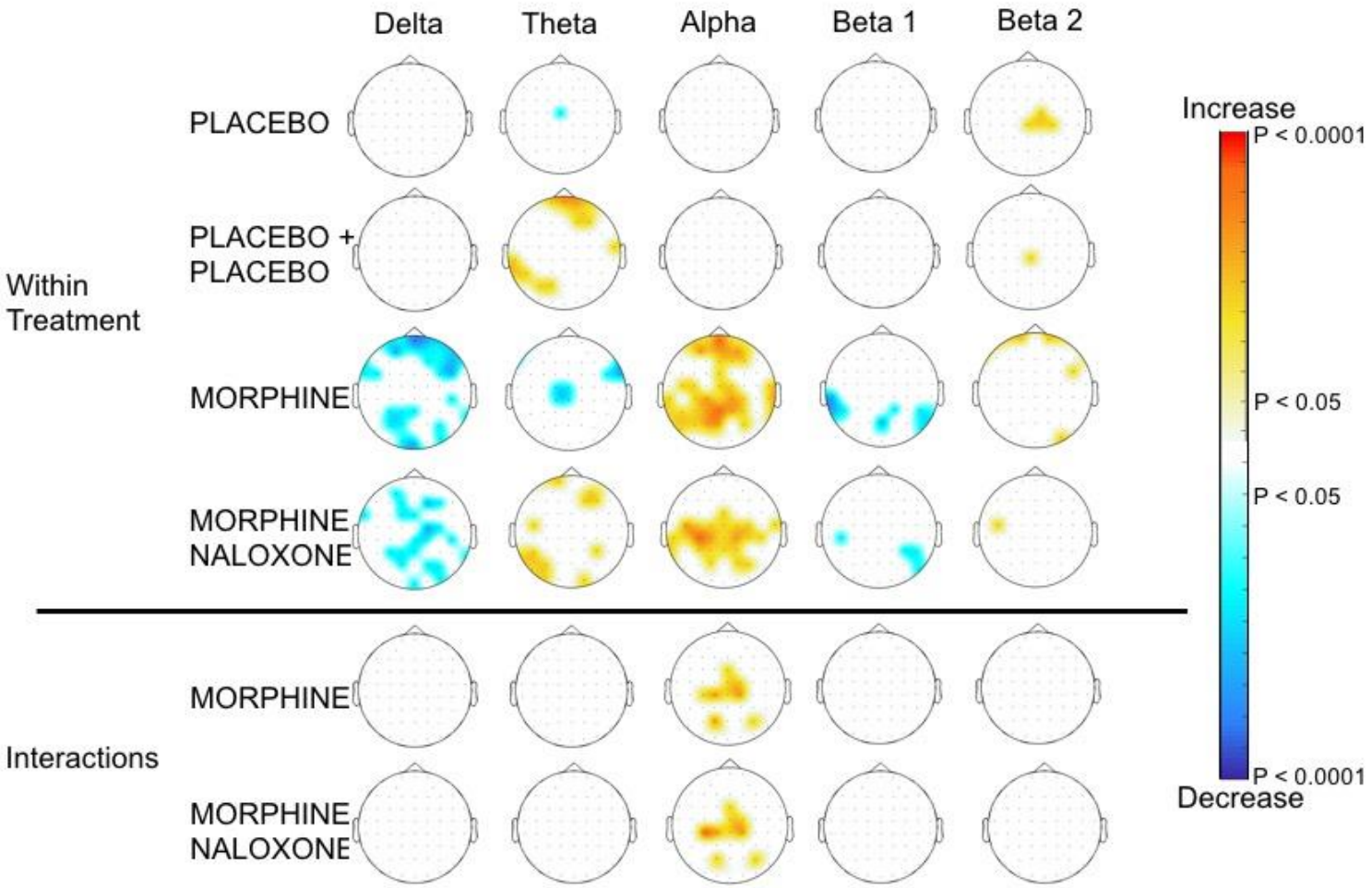

Figure 4. Topographical plots showing statistically significant differences for resting EEG. Black dots depict the electrodes. Top part of the figure shows within treatment differences and bottom part of the figure shows interactions. White color means there was no statistically significant difference, whereas blue color shows significant decrease and red color shows significant increase. ***There were some changes due to morphine in resting EEG. However, none of these changes were reversed by naloxone and thus they are likely not opioid specific.***

	BASELINE PLACEBO	PLACEBO	PLACEBO + PLACEBO	BASELINE MORPHINE	MORPHINE	MORPHINE + NALOXONE
Sensory, Reflex and Pain Tolerance Thresholds						
Foot Sens. (mA)	2.8±0.2	2.9±0.1	3.1±0.2	3.1±0.2	3.4±0.2	3.3±0.2
NWR (mA)	9.9±1.8	10.2±1.3	11.2±1.6*	9.5±1.5	11.4±1.7*	13.8±1.7***
Med. Sens. (mA)	2.5±0.1	2.6±0.2	2.6±0.2	2.4±0.1	2.7±0.2	2.7±0.2
Med. Refl. (mA)	8.5±0.6	9.1±0.4	9.4±0.6*	8.5±0.4	9.3±0.4	9.2±0.4
Heat PTT (°C)	48.4±0.4	48.0±0.5	48.4±0.4	48.5±0.3	48.5±0.4	***47.7±0.4****
Elec. PTT (mA)	18.8±2.1	19.1±2.3	18.8±2.3	17.7±1.9	***23.4±3.0****	18.7±2.2
Press. PTT (kPa)	7504±506	***6735±435****	***6434±407****	7506±445	***8678±843****	***6572±416****
Unpleasantness and Pain Scores						
NWR*1.3 Unp.	2.9±0.4	2.8±0.4	2.7±0.4	3.0±0.3	2.3±0.3*	2.9±0.4
NWR*1.6 Unp.	3.7±0.4	3.6±0.5	3.6±0.4	3.7±0.4	2.9±0.4**	3.7±0.4
NWR*2.0 Unp.	4.6±0.4	4.6±0.4	4.5±0.4	4.6±0.5	***3.6±0.5****	4.7±0.4
NWR*1.3 Pain	1.3±0.3	1.3±0.3	1.5±0.3	1.7±0.4	1.2±0.3	1.6±0.4
NWR*1.6 Pain	1.9±0.3	1.7±0.3	2.1±0.4	2.1±0.4	1.6±0.3	2.5±0.4
NWR*2.0 Pain	2.5±0.4	2.7±0.4	3.0±0.4	3.0±0.5	2.5±0.4	3.2±0.5
2°Water Unp. 40s	6.8±0.5	7.1±0.4	7.1±0.4	6.7±0.4	***5.4±0.4****	6.7±0.3
2°Water Unp. 80s	7.9±0.3	8.0±0.4	8.2±0.3	7.7±0.3	***6.3±0.4****	8.1±0.3
2°Water Unp. 120s	7.3±0.4	7.4±0.5	7.4±0.4	7.1±0.4	***5.8±0.4****	7.2±0.4
2°Water Pain 40s	5.4±0.3	5.3±0.3	5.8±0.3	5.3±0.4	4.4±0.5**	5.4±0.4
2°Water Pain 80s	6.5±0.3	6.7±0.4	7.0±0.3	6.4±0.3	***5.4±0.5****	6.7±0.3
2°Water Pain 120s	6.4±0.4	6.5±0.4	6.7±0.4	6.0±0.4	***5.0±0.6****	6.4±0.4

Table 2. Thresholds and sensory ratings. The highlighted cells represent the values which showed statistically significant changes. Blue color depicts decrease and orange color depicts increase. The parameters which are colored show change within treatment. The parameters which are in bold and italics had significant interactions. PLACEBO = first placebo infusion; PLACEBO + PLACEBO = second placebo infusion together with the first one; MORPHINE = lone morphine infusion; MORPHINE + NALOXONE = naloxone infusion together with morphine. Abbreviations: Sens. = Sensory; NWR = Nociceptive withdrawal reflex; Med. = Median; Elec. = Electrical; PTT = Pain tolerance threshold; Press. = Pressure; Unp. = Unpleasantness. *p<0.05, **p<0.01, ***p<001. It can be seen that the main changes due to morphine , which were reversed by naloxone are: ***Increase in electrical tetanic stimulation pain tolerance threshold, increase in bone pressure pain tolerance threshold, decrease in unpleasantness ratings of NWR stimulations and decrease in pain and unpleasantness ratings of cold-pressor test.***

	BASELINE PLACEBO		PLACEBO		PLACEBO + PLACEBO	
	LATENCY	AMPLITUDE	LATENCY	AMPLITUDE	LATENCY	AMPLITUDE
P9-Erb	9.5±0.6	2.7±1.2	9.5±0.6	2.6±1.2	9.5±0.7	2.6±1.1
N10-Erb	11.0±0.7	7.8±3.0	11.0±0.7	7.5±3.3	10.9±0.7	7.5±2.9
P11-Spinal	11.2±0.8	1.3±0.7	11.3±0.8	1.4±0.6	11.1±0.7	1.4±0.9
N13-Spinal	14.2±1.0	2.2±0.9	13.9±1.1	2.2±0.7	13.9±1	2.2±1.2
	BASELINE MORPHINE		MORPHINE		MORPHINE + NALOXONE	
	LATENCY	AMPLITUDE	LATENCY	AMPLITUDE	LATENCY	AMPLITUDE
P9	9.5±0.6	2.6±1.4	9.5±0.6	2.5±1.4	9.5±0.5	2.6±1.3
N10	11.0±0.7	7.5±3.6	11.0±0.7	7.8±3.8	11.0±0.7	7.6±3.6
P11	11.0±0.6	1.3±0.4	11.1±0.7	1.3±0.5	11.1±0.7	1.1±0.4
N13	13.8±0.7	2.3±0.9	14.1±0.8	2.4±0.6	14.0±0.8	2.2±0.6

Table 3. Spinal evoked potential responses to median nerve stimulations. PLACEBO = first placebo infusion; PLACEBO + PLACEBO = second placebo infusion together with the first one; MORPHINE = lone morphine infusion; MORPHINE + NALOXONE = naloxone infusion together with morphine. ***There were no changes of spinal evoked potentials in either experimental arm.***

	Latencies (ms)					
	BASELINE PLACEBO	PLACEBO	PLACEBO + PLACEBO	BASELINE MORPHINE	MORPHINE	MORPHINE + NALOXONE
P14	15.4±0.7	15.5±0.9	15.3±0.8	15.5±0.6	15.3±0.8	15.6±0.7
N20	20.7±1.1	20.6±0.9	20.6±1.0	20.8±0.8	20.9±1.1	20.7±0.8
P25	25.7±1.8	25.6±1.6	25.6±1.8	25.4±1.6	25.7±1.7	26.0±2.2
N30	34.2±3.4	33.9±3.5	34.1±3.2	35.0±3.1	34.7±3.2	34.7±3.5
P45	43.0±3.1	42.9±3.4	42.9±3.2	42.8±3.3	43.7±3.4	44.2±3.5
N60-80	61.2±7.7	63.3±8.6	61.9±8.0	62.5±7.9	62.9±6.6	63.8±8.1
P100-120	106.5±25.5	108.6±26.7	106.6±26.6	104.2±26.0	107.9±24.2	106.3±26.6
	Amplitudes (μV)					
	BASELINE PLACEBO	PLACEBO	PLACEBO + PLACEBO	BASELINE MORPHINE	MORPHINE	MORPHINE + NALOXONE
P14	0.6±0.2	0.6±0.3	0.7±0.2	0.6±0.3	0.7±0.3	0.7±0.3
N20	1.1±0.5	1.2±0.6	1.2±0.6	1.2±0.4	1.2±0.6	1.2±0.6
P25	1.4±0.7	1.5±0.9	1.5±1.0	1.4±0.9	1.5±1.2	1.4±0.9
N30	3.2±2.1	3.3±2.0	3.2±2.0	3.1±2.0	3.0±2.2	3.1±2.0
P45	1.8±1.5	1.8±1.5	1.7±1.3	1.9±1.3	1.9±1.4	2.1±1.8
N60-80	2.1±1.0	2.1±0.9	2.2±0.9	2.1±0.7	2.3±0.8	2.3±1.1
P100-120	2.5±1.4	2.5±1.2	2.5±1.2	2.4±1.4	2.3±1.1	2.5±1.1

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Table 4. Cortical evoked potential responses to median nerve stimulations. PLACEBO = first placebo infusion; PLACEBO + PLACEBO = second placebo infusion together with the first one; MORPHINE = lone morphine infusion; MORPHINE + NALOXONE = naloxone infusion together with morphine. ***There were no changes of the cortical evoked potentials to median nerve stimulations in either experimental arm.***

	BASELINE PLACEBO	PLACEBO	PLACEBO + PLACEBO	BASELINE MORPHINE	MORPHINE	MORPHINE + NALOXONE
TA AUC (EMG) @ 1.3	15.8±2.9	13.8±3.0	18.6±5.9	18.8±3.5	13.9±3.9*	20.0±5.1
TA AUC (EMG) @ 1.6	25.3±5.6	20.6±5.4	25.8±6.8	29.6±5.7	20.9±4.8**	30.6±6.7
TA AUC (EMG) @ 2.0	31.9±7.1	34.1±7.3	34.8±8.7	40.7±8.1	28.1±6.4**	43.4±9.3
LATENCY 1 Cz (ms)	115.4±3.3	111.8±3.7	113.5±4.0	113.2±3.4	111.0±4.0	113.8±4.4
AMP 1 Cz (μV)	20.9±1.8	19.2±1.7*	19.5±1.9	21.3±1.8	19.9±2.0*	20.0±1.8
LATENCY 2 Cz (ms)	257.7±8.4	253.4±7.6	251.9±8.1	261.1±8.1	258.8±8.6	258.1±8.3
AMP 2 Cz (μV)	27.1±1.7	26.2±1.7	25.3±2.1^	27.4±1.8	24.6±2.1**	26.2±2.0
ACC STRENGTH	174.2±13.6	161.6±13.6	155.9±14.3*	167.1±15.1	147.8±14.2**	167.0±15.5
FRONTAL STRENGTH	90.4±6.2	89.8±6.5	92.3±6.4	86.4±6.3	84.3±7.1	83.0±6.5
INSULA R STRENGTH	71.3±4.3	72.1±4.9	67.1±5.0	68.2±5.3	***61.1±4.6****	70.5±6.2
INSULA L STRENGTH	74.7±4.5	75.3±5.5	73.3±4.6	74.5±5.7	69.9±5.9	74.6±5.3

Table 5. Reflex EMG and EP results. It can be seen that morphine reduces the magnitude of EMG at tibialis anterior and it also reduces the magnitude of the second main peak at Cz electrode as well as ACC and right insula source strengths. All of these changes were reversed by naloxone. PLACEBO = first placebo infusion; PLACEBO + PLACEBO = second placebo infusion together with the first one; MORPHINE = lone morphine infusion; MORPHINE + NALOXONE = naloxone infusion together with morphine. Abbreviations: TA=tibialis anterior, AUC=area under curve, ACC=anterior cingulate cortex; *$P < 0.05$, **$P<0.01$, ^$P=0.05$. Bold and italic=significant interaction, otherwise only significant within treatment effect. ***It can be seen that the main changes due to morphine which were reversed by naloxone are decreases in EMG responses to NWRs and decreased strength of right insular activity.***

	BASELINE PLACEBO	PLACEBO	PLACEBO + PLACEBO	BASELINE MORPHINE	MORPHINE	MORPHINE + NALOXONE
				Offset Analgesia		
T2 max score	7.0±0.5	6.8±0.5	7.3±0.4	7.2±0.4	6.5±0.5	6.8±0.5
T3 min score	3.9±0.6	3.0±0.5	3.5±0.5	3.8±0.4	2.5±0.5*	2.4±0.5**
VAS % change	-46±7.4%	-52±7.6	-50±7.1	-46±6.4	-61±6.4**	-64±6.5**
				Conditioning Pain Modulation		
El % change 90s	+10.1±5.4	+17.9±4.2	+13.6±4.0	+14.6±4.8	+5.2±6.4	+6.4±6.4
Pr % change 90s	+11.8±4.2	+9.3±4.0	+13.6±3.5	+12.2±3.4	+5.0±4.0	+6.8±3.9
El % change 150s	+7.8±5.7	+17.6±4.1	+13.4±3.8	+14.3±5.4	+3.9±7.2	+4.5±6.2
Pr % change 150s	-5.4±2.8	-2.5±2.7	3.8±4.0	-0.6±4.7	-7.8±6.5	-2.2±3.7

Table 6. Offset analgesia and conditioning pain modulation data. It can be seen that both morphine and naloxone increased the magnitude of offset analgesia within the active treatment arm. Interactions, however, were not significant. Although there was a trend of decreased magnitude of CPM, neither drug significantly modified the CPM. PLACEBO = first placebo infusion;

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PLACEBO + PLACEBO = second placebo infusion together with the first one; MORPHINE = lone morphine infusion; MORPHINE + NALOXONE = naloxone infusion together with morphine. Abbreviations: EI – electrical tetanic stimulation PTT threshold; Pr – bone pressure PTT. *P<0.05, **P<0.01. ***Morphine increased the magnitude of offset analgesia, but this was not reversed by naloxone and hence this phenomenon is likely not opioid dependant. Morphine had no effect no conditioning pain modulation.***

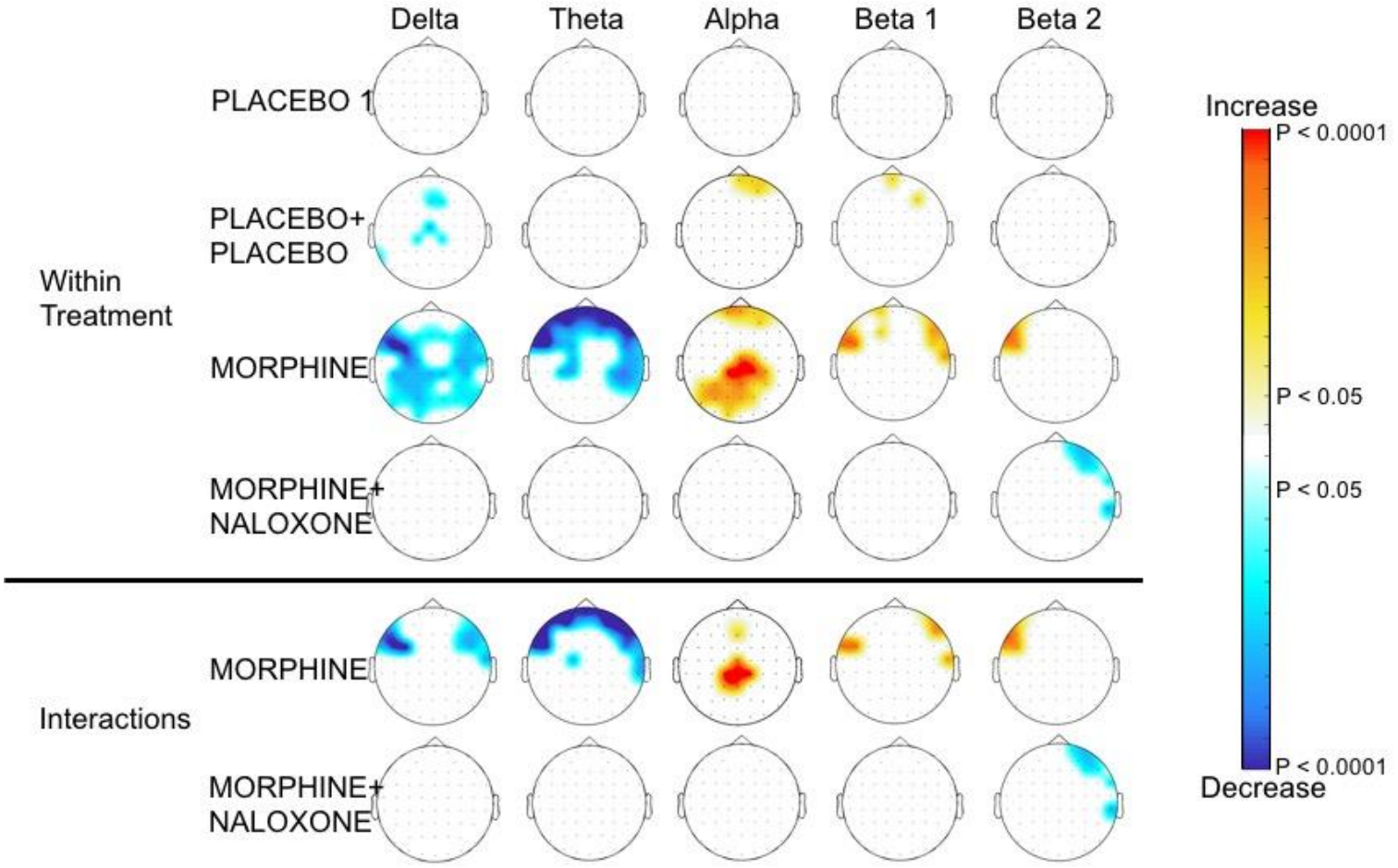
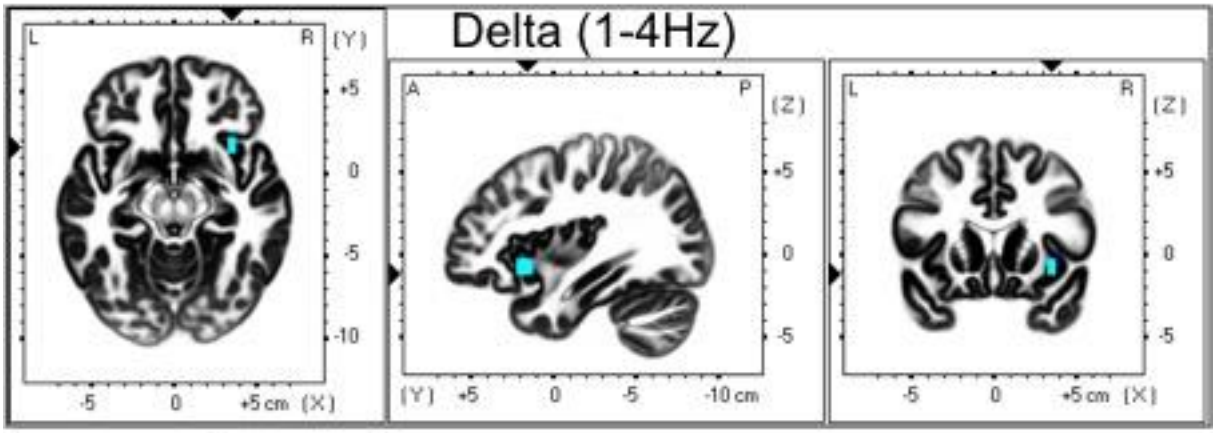
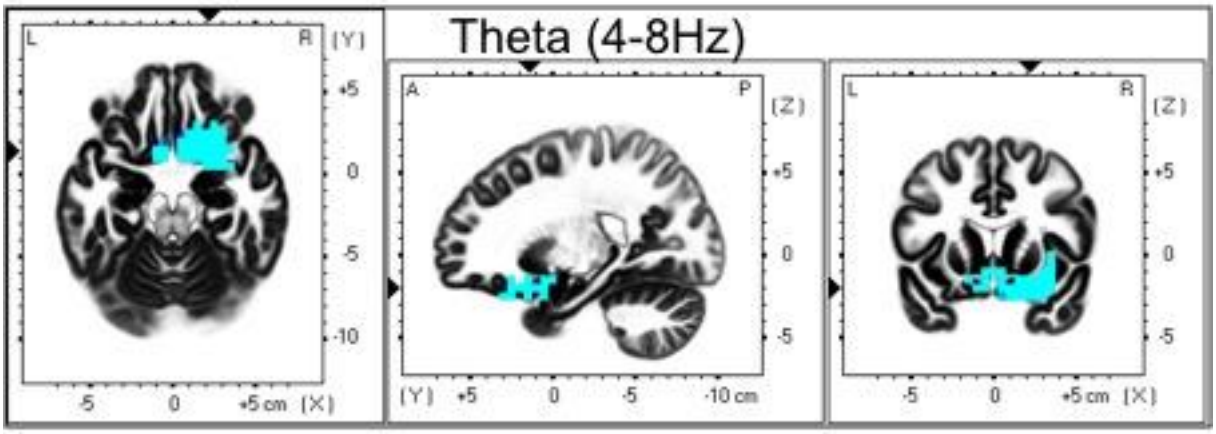


Figure 5. Topographical plots showing statistically significant differences for cold-pressor EEG. Black dots depict the electrodes. Top part of the figure shows within treatment differences and bottom part of the figure shows interactions. White color means there was no statistically significant difference, whereas blue color shows significant decrease and red color shows significant increase. ***It can be seen that morphine decreased the surface cortical EEG responses in delta and theta bands, whereas it increased the surface EEG activity in alpha frequency band. All which were reversed by naloxone.***


Delta (1-4Hz)
L
R
(Y)
+5
0
-5
-10
-5
0
+5 cm (X)
A
P
(Z)
(Y) +5 0 -5 -10 cm
L
R
(Z)
-5
0
+5 cm (X)


Theta (4-8Hz)
L
R
(Y)
+5
0
-5
-10
-5
0
+5 cm (X)
A
P
(Z)
(Y) +5 0 -5 -10 cm
L
R
(Z)
-5
0
+5 cm (X)

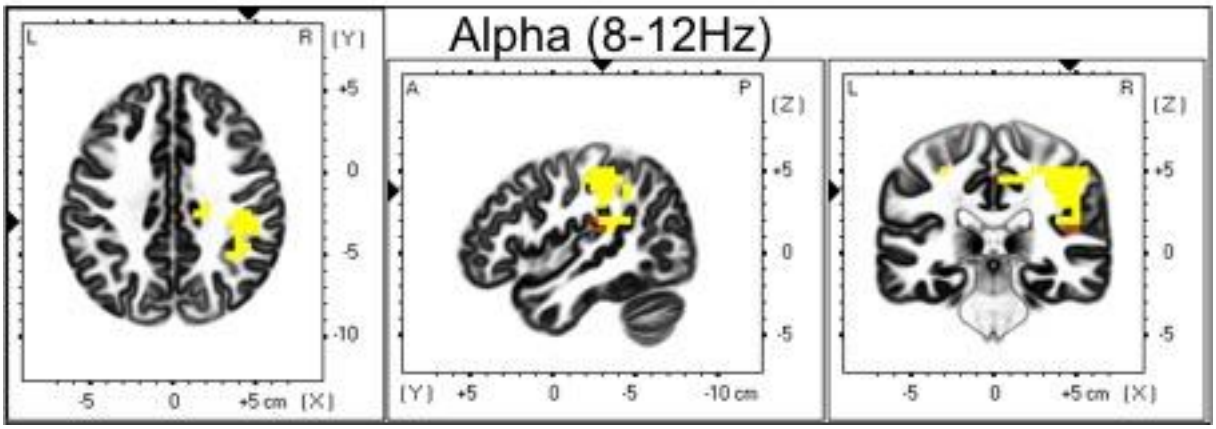

Alpha (8-12Hz)
L
R
(Y)
+5
0
-5
-10
-5
0
+5 cm (X)
A
P
(Z)
(Y) +5 0 -5 -10 cm
L
R
(Z)
-5
0
+5 cm (X)

Figure 6. Cold pressor LORETA solution in the morphine arm. Blue color depicts significant decreases and red color depicts significant increases. Placebo, placebo + placebo and morphine + naloxone data are not shown because there were no statistically significant differences due to either treatment. ***These results are in line with the surface EEG response where decreases in delta and theta and increase in alpha band were seen. Here we know specifically which brain areas are responsible for the changes seen in the surface EEG.***

	40 seconds		80 seconds	
	R*	***P*-value**	**R***	***P*-value**
Delta spectral activity	0.44	< 0.001	0.40	< 0.01
Alpha spectral activity	-0.51	< 0.001	-0.56	< 0.0001
Alpha LORETA	-0.48	< 0.001	-0.52	< 0.0001
Parietal Lobe	-0.46	< 0.001	-0.50	< 0.001
Insula	-0.35	< 0.01	-0.38	< 0.01
Cingulate Gyrus	-0.47	< 0.001	-0.53	< 0.0001
Temporal Lobe	-0.37	< 0.01	-0.39	< 0.01
Frontal Lobe	-0.44	< 0.001	-0.53	< 0.0001

Table 7. Cold-Pressor EEG spectral and LORETA correlations to cold-pressor pain scores in the morphine arm. Spectral activity represents the surface EEG activity and LORETA is modelling of underlying neuronal activity. *Spearman's Rho. ***Changes seen in EEG responses to tonic pain are correlated to the pain relief due to morphine.***