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Acupuncture – Deep pain with an autonomic dimension?

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Abstract

Acupuncture point Pc6, located above the median nerve, has been shown to be effective in treating nausea and vomiting. Its stimulation frequently causes a heart rate reduction. The mechanism behind this autonomic reaction has not been clarified, so far. We combined brainstem-sensitive functional magnetic resonance imaging with heart rate recording and time-resolved rating of the needling sensation to measure neuronal correlates of sensations and autonomic reactions during acupuncture. On the cortical level, needling sensation activated typical pain-related areas, of which the ventromedial and dorsolateral prefrontal cortex and perigenual anterior cingulate cortex were further involved in mediating the heart rate response. In the brainstem, needling sensation activated nuclei of the descending pain control system, in which a network of hypothalamus, periaqueductal gray, rostral ventromedial medulla, and ventrolateral medulla was identified as the source of the heart rate changes. Our findings indicate that acupuncture may be a special pain stimulus, whose autonomic concomitants could explain its non-analgesic effects and in some cases even have a therapeutic potential.

Keywords: acupuncture, functional magnetic resonance imaging, brainstem, autonomic nervous system, pain
Introduction

Acupuncture is an ancient Asian medical intervention, where needles are inserted at predefined points of the body to apply therapeutic stimulation. Its clinical effectiveness is still subject to heavy debates (Ernst 2006; Madsen et al. 2009). While the search for an underlying mechanism of acupuncture's analgesic effects has led to a plethora of hypotheses (Zhao 2008), some of the best-researched effects are of non-analgesic nature. A frequent and astonishing finding of acupuncture experiments in animals and humans is a drop in heart rate and blood pressure, when stimulating acupuncture points above the median nerve at the wrist (Nishijo et al. 1997; Uchida et al. 2010; Li & Longhurst 2010; Li et al. 2009). This autonomic effect of acupuncture resembles a so-called passive coping response evoked by deep pain consisting of sympathoinhibition and hyporeactive immobility (Lewis 1942, Lumb 2004), while superficial pain usually evokes an active coping response consisting of sympathoexcitation, and increased motor activity (Lumb 2004).

According to standard textbooks on acupuncture, points in this area are frequently used to treat heart pain, palpitations, disorders of heart rhythm, asthma, nausea and vomiting (Deadman et al. 1998). Effectiveness of acupuncture in treating the two latter indications even seems to be of clinical relevance (Ezzo et al. 2006; A. Lee & Fan 2009). A common central mechanism behind these seemingly unconnected indications may lie in an influence that acupuncture exerts on the autonomic nervous system (ANS). This view is supported by acupuncture studies that have measured heart rate variability in human subjects showing a change in sympatho-vagal balance towards vagal predominance (Nishijo et al. 1997; Huang et al. 2005). Similar results have been obtained by electrogastrography studies, which have repeatedly shown a reduction of sympathetically-induced gastric tachyarrhythmia and an increase of normal gastric slow waves by acupuncture in human subjects (Yin & J. D. Z. Chen 2010).

Animal studies have identified a number of mesencephalic and brainstem nuclei putatively involved in mediating such autonomic effects (Li et al. 2009). These include hypothalamic areas (Hyp), like
the Ncl. arcuatus (also: infundibular nucleus), the ventrolateral periaqueductal gray (vlPAG), and the rostral ventrolateral medulla (rVLM) in the brainstem.

We hypothesized that acupuncture-induced heart rate changes in humans are the autonomic concomitant of the deep pain stimulation by the needle and are mediated by the same autonomic network of mesencephalic and brainstem nuclei that have been found in animal experiments. To test this hypothesis, we combined a brainstem-sensitive whole-brain functional magnetic resonance imaging (fMRI) sequence with time-resolved rating of the needling sensation (Napadow et al. 2009) and parallel heart rate recording.

Materials and Methods

23 healthy subjects (15 male) participated in the study. The mean age was 27.2 ± 7.5 years (S.D.). All participants gave written informed consent following the guidelines of the ethics committee of the Goethe University Frankfurt/Main, Germany, who had approved the study. All subjects were right-handed as assessed by the Edinburgh inventory (Oldfield 1971). All subjects were healthy as assessed by an interview and clinical examination. Data from four subjects (3m/1f) had to be discarded due to withdrawal (2 subjects) and problems with the pulse recording (2 subjects). Subjects were told that they participated in a study to investigate the underlying mechanisms of acupuncture. For the whole experimental procedure the reader is referred to Figure 1. Prior to the actual fMRI experiment, subjects were trained to rate the intensity of the needling sensation on a visual analogue scale (VAS). Instructions were given in the MR preparation room, and one needle was placed into acupoint “Li11 (Quchi)” at the lateral end of the transversal cubital crease (Deadman et al. 1998) and stimulated by twisting at ~1Hz. This location was chosen, as it is relatively easy to elicit a needling sensation here. Furthermore, indications of this point are largely disjunct from that of Pc6. Subjects were asked for their sensations during stimulation. When they reported a “dull”, “aching”, or “pressing” sensation, they were told to rate the intensity of these
sensations on the VAS, which ranged from 0 (“non-perceptible”) to 100 (“maximally tolerable intensity”). The VAS was implemented in Presentation® (Neurobehavioural Systems, Inc., Albany, CA) and consisted of a simple bar of uniform color without numbers and with a vertical line indicating the intensity. The line was moving slowly to the right and subjects had to press a button, as soon as the line reached the position indicating their perceived intensity of the acupuncture sensation. The initial position of the line was changed every time to avoid bias, which induced the natural jitter in presentation frequency (as the line's initial position could be close of far away from the value that the subject intends to rate). When the line reached 100 it jumped back to 0 with a short delay of 0.5 seconds. In addition, each presentation of the VAS was followed by 5 seconds without presentation. This on-line rating method has first been introduced by Napadow et al. in a slightly different form (Napadow et al. 2009).

The fMRI measurements consisted of two consecutive experimental runs. The onset of the fMRI measurements was delayed until the subjects reported that there were no residual sensations due to the acupuncture test stimulation. The minimum waiting period was 10 minutes. In the first run, comprising 93 scans, subjects successively performed three motor tasks in a blocked design of 20 seconds block length to activate well-known nuclei of the brainstem. Grimacing was used for the facial nucleus, jaw-clenching for the trigeminal motor nucleus, and swallowing for the ambiguus nucleus. This first run had two main objectives: To test the sensitivity of the fMRI protocol and to ease the later identification of brainstem activations by their relative position to those of the well-known nuclei. Results have been published previously by our group (Beissner et al. 2011).

In the second run, which comprised 160 scans, acupuncture stimulation was applied to acupoint “Pc6 (Neiguan)”, which is located about five centimeters proximal to the distal crease of the wrist of the left arm, between the tendons of palmaris longus and flexor carpi radialis muscles (Deadman et al. 1998). The needle was inserted perpendicularly to the skin at a depth of about one centimeter and twisted for stimulation about once per second. The moment of insertion was one minute after
starting the fMRI measurement and indicated to the investigator (who also applied the acupuncture) by an auditory signal via headphones. The VAS was presented to the subjects using video goggles. A fixation cross was presented during the intervals between the ratings. Stimulation intensity was adjusted to the subjects’ perception, i.e. the needle was twisted, whenever the rated intensity fell below 20/100 on the VAS. This was again indicated to the investigator by an auditory feedback via headphones. Subjects were not informed about the intensity-adjusted stimulation to prevent deliberate higher ratings by the subjects to avoid stimulation. The individual acupuncture sensation time-courses were later used as predictor variable in the fMRI data analysis. To avoid differences in the mode of stimulation, all acupuncture interventions were carried out by the same qualified acupuncturist (F.B.) applying the same stimulation technique.

Data acquisition

Measurements were performed on a 3T MR head scanner (Allegra; Siemens Medical Solutions, Erlangen, Germany) equipped with a 4-channel head transmit/receive coil. A single-shot dual-echo EPI sequence (Beissner et al. 2010) was gated to the subject’s pulse, which was measured using the vendor-supplied photoplethysmograph. Measurement parameters were: TE$_1$ = 28 ms and TE$_2$ = 50 ms, TR$_0$=3.6 s, FOV: 192×192 mm$^2$, matrix size: 64 × 64, in-plane resolution 3×3 mm$^2$, 40 axial slices in ascending, non-interleaved order, slice thickness: 2.5 mm, interslice gap: 1.25 mm, yielding an effective spatial resolution of 3.75 mm in slice direction. Readout bandwidth: 3551 Hz/pixel, trapezoidal readout gradients with a ramp time of 100 μs and a flat top duration of 140 μs, echo spacing: 340 μs, duration of each echo train comprising 64 phase encoding steps: 21.76 ms. These parameters allowed us to exploit the noise reduction of cardiac gating in the brainstem and still acquire whole-brain images.

A structural MR image (MPRAGE, FOV: 256×256 mm$^2$, matrix size: 256 × 256, 1mm isotropic resolution, 144 slices) was acquired after the functional runs.
Data analysis – VAS data

Rating time-courses from the VAS were processed using MATLAB® (MathWorks, Natick, MA). Time series were interpolated and resampled to match the individual mean repetition (TR) time of the fMRI measurement. For correlation analysis with the pulse data, time series were resampled to 0.25 Hz, the approximate mean repetition frequency of the fMRI measurements over all subjects.

Data analysis – pulse data

Raw pulse and respiration readings were preprocessed using a combination of AcqKnowledge® (BIOPAC Systems, Inc., Aero Camino Goleta, CA), Kubios HRV® (Biosignal Analysis and Medical Imaging Group, University of Kuopio, Finland) and MATLAB®. After heart beat detection, inter beat intervals (IBIs) were calculated from the pulse data. After artefact correction, the heart rate time series (in beats per minute, bpm) were calculated by successively averaging over five consecutive IBIs. Like the VAS readings, heart rate time series were subsequently resampled to 0.25 Hz for correlation analysis. For the respiration data, local maxima were detected to calculate the respiration rate. The root mean square of successive differences (RMSSD) as a measure for vagal activity was calculated from the heart rate signal using the formula

\[ RMSSD = \sqrt{\frac{1}{n} \sum (IBI_i - IBI_{i-1})^2} \]  
(Task-Force 1996).

Correlation coefficients were calculated for the VAS and the time-courses of heart rate, respiration rate and RMSSD after being z-transformed. P-values were computed for the null hypothesis of zero correlation, and values below 0.05 were considered significant.

Data analysis – fMRI data

Image time series of the fMRI measurements were preprocessed using FSL 4.1 (FMRIB, Oxford). For motion correction, transformation parameters were derived from the images acquired at short TE. First- and second-echo time series were corrected with the same parameters. After spatial
smoothing with an isotropic Gaussian kernel of 4.5mm FWHM, both time series were combined using the equation

\[ T_2^* = \frac{TE_2 - TE_1}{\ln(S_1/S_2)} \]

for each voxel, where \( S_{1/2} \) is the intensity at the first/second echo time. The resulting \( T_2^* \) time series were free of unwanted \( T_1 \)-related signal fluctuations caused by the variable TR due to cardiac gating. Finally, images were masked with a brain extraction mask.

First-level statistical analysis was carried out using FSL. After high-pass filtering (cut-off: 60 s for the first, 120s for the second run) and pre-whitening, a GLM-based analysis was run. The subject's time-course of acupuncture sensation intensity was convolved with the canonical haemodynamic response function and used as predictor variable in the analysis. Mean TR values were used instead of the actual repetition times (which were variable due to cardiac gating).

Before group-level analysis, contrast and variance images of the first-level analyses were normalized to the MNI152 template (Montreal Neurological Institute) using a previously described approach (Beissner et al. 2011) based on FSL's normalization algorithm. Transformation parameters were estimated from the anatomical high resolution images. The mean functional image (first echo) was then coregistered to the anatomical image. The resulting transformation was applied to the contrast and variance images, which were resampled to 2mm isotropic resolution. For the results of coregistration and normalization see Supplementary Figure 1.

A mixed-effects analysis was carried out using FSL’s FLAME (Beckmann et al. 2003; Woolrich et al. 2004). Two one-sample t-tests were calculated. In the first t-test all subjects were weighted equally, thus asking for areas commonly activated by the acupuncture stimulation. In the second t-test subjects were weighted by their correlation coefficients between acupuncture sensation and heart rate, testing for areas that play a role in acupuncture’s effect on heart rate or – more general – on autonomic regulation. For the whole-brain analyses, results were corrected for multiple comparisons using cluster correction (Friston et al. 1994) with a voxel-inclusion threshold of \( z = 2.3 \) and a corrected p-value of 0.05. For the brainstem analyses, results were thresholded at an uncorrected p < 0.005 (\( z < 2.58 \)) in analogy to earlier brainstem fMRI studies (Eippert et al. 2009).
Results

To assess the effects of acupuncture stimulation, we recorded the exact time course of the needling sensation in every subject using time-resolved rating on a visual analogue scale (VAS) (Napadow et al. 2009). Ratings showed a high inter-individual variability (Figure 2, black curves). In four subjects (number 2, 9, 13, and 16), needle stimulation had to be maintained throughout the entire experiment as their ratings reached 20/100 points on the VAS only during constant stimulation. For all other subjects, sensation intensity exceeded 20/100 points for several seconds to minutes without further stimulation. Even periods with no stimulation showed spontaneous changes in needling sensation intensity in the order of 50/100 points in some subjects. Eleven out of 19 subjects showed significant (p<0.05) negative temporal correlations of needling sensation intensity and heart rate time-courses, i.e. heart rates were lower for stronger perceived needling sensation (Figure 2, blue curves). The average correlation coefficient r of all subjects was -0.225 ± 0.065 (S.D.) indicating a small effect, which was highly significant on group level (t(18)=-3.485, p=0.003). Since most of the high-frequency component of heart rate variability reflects respiratory sinus arrhythmia (Katona & Jih 1975), which may be influenced by respiratory frequency (Pöyhönen et al. 2004; Sanderson et al. 1996), we also looked for acupuncture-related changes in respiration. Respiration rates correlated negatively in 4 subjects, albeit, not reaching significance on group level (t(18)=-1.774, p=0.093). RMSSD time courses, indicating vagal activity, showed small but consistent positive correlations with needling sensation time courses that were significant in 3 single subjects and on group level (t(18)=2.1964, p=0.041). Thus, the overall effect of acupuncture stimulation on heart rate can be described as a change in sympatho-vagal balance towards vagal predominance.

Cortical BOLD responses

fMRI group results showed significant (p<0.05, cluster corr.) BOLD signal increases in the
following areas, when the individual time courses of needling sensation intensity were used as predictors: Primary and secondary somatosensory cortex (postcentral gyrus, supramarginal gyrus, and superior parietal lobule), anterior mid-cingulate cortex (ACC), inferior, middle and superior frontal gyrus (IFG / MFG / SFG), insula extending to the frontal operculum and orbitofrontal cortex, lateral occipital cortex (LOC), and dorsolateral prefrontal gyrus (dlPFC). Deactivations were found in one large cluster comprising ventromedial prefrontal and orbitofrontal cortices as well as perigenual and subgenual cingulate cortex (vmPFC / pgACC) (Table 2, Figure 3).

When first-level results were weighted with the individual heart rate responses (represented by the correlation coefficients from Table 1), only two of the previously observed regions (vmPFC / pgACC and dlPFC) were still found significant (Table 2, Figure 3). The weighting approach asks for areas that are activated by the needling sensation and are in addition involved in the autonomic response. Because of the predominantly negative correlation coefficients used for the weighting, activation in this analysis meant reduced neuronal activity in subjects with a strong heart rate response and vice versa. As shown in Table 2, the vmPFC / pgACC cluster was about 50 percent larger (in terms of voxel count) in the weighted analysis. Both clusters (vmPFC / pgACC and dlPFC) changed signs in accordance with the above considerations.

In addition to these clusters, two new areas were discovered that showed significant BOLD signal changes: The lateral occipital and fusiform gyrus (deactivation) and the head of the caudate nucleus extending to the subcallosal cortex (activation) (Table 2, Figure 3).

**Brainstem BOLD responses**

Significant (p<0.005, uncorr.) BOLD signal changes in the brainstem were observed in the locus coeruleus (LC), while no deactivations were found (Figure 4, top row, b).

When first-level results were weighted with the individual heart rate responses as shown for the cortex, the LC cluster changed its sign from activation to deactivation. Furthermore, two new
cluster emerged, one of activation in the hypothalamus (Ncl. arcuatus) and one of deactivation comprising the ventrolateral medulla (VLM) and the nucleus ambiguus (NAmb) (Figure 4, second row, a-c).

All activations are subsumed on the right-hand side of Table 2. The localizations of the LC and the hypothalamic clusters were confirmed using an LC template (Keren et al. 2009) and a ROI based on an MRI atlas of the human hypothalamus (Baroncini et al. 2012) (see Supplementary Figure 2).

Discussion

In this study, we have presented indications that heart rate changes during acupuncture stimulation at acupoint Pc6 are the consequence of a shift in sympatho-vagal balance towards vagal predominance, which is mediated by a circumscribed network of cortical and subcortical areas. Judging from the correlation coefficients in Table 1, the observed shift is not induced by changes in respiratory frequency. The increase of vagal activity was found to be rather small as evidenced by the weak correlation of RMSSD and needling sensation time courses. Thus, the most likely explanation is a reduction of sympathetic nervous system activity, which is also corroborated by the observed involvement of the ventrolateral medulla, a key brainstem center of sympathetic control (see below).

On the neurophysiological level, acupuncture activated cortical areas typically involved in pain perception (Tracey & Mantyh 2007), like the primary and secondary somatosensory cortex (SI / SII), the medial anterior cingulate cortex (ACC), the insula, and the dorsolateral prefrontal cortex (dLPFC). As we have recently shown, activation of pain-related areas in the brain is one of the few consistent findings of acupuncture-fMRI studies so far (Beissner 2011). Thus, despite a plethora of other hypotheses, acupuncture in the end may turn out to be but a special deep pain stimulus.

Further evidence for this view is provided by strong similarities between the needling sensation evoked by acupuncture and sensations reported during deep pain stimulation. Henderson et al.
compared pain sensations evoked by superficial and deep injection of hypertonic saline (Henderson et al. 2006). They found that “heavy”, “aching”, “cramping”, “throbbing”, and “gnawing” are the descriptors most frequently chosen to describe deep pain. A comparison with the acupuncture setting shows that very similar descriptors are being used to describe needling sensation (Hui et al. 2007; Hui et al. 2011). Another feature is that deep pain in contrast to superficial pain tends to radiate from the point, where it is elicited (Lewis 1942; Burton et al. 2009), a finding reminiscent of the radiating and spreading character of the needling sensation. The constant direction of such radiating sensations may have inspired early Chinese physicians in their development of the meridian concept (Longhurst 2010).

Although cortical responses to acupuncture stimulation were dominated by activations, the largest single cluster was a deactivation located in the ventromedial prefrontal cortex (vmPFC), which included parts of the perigenual anterior cingulate cortex (pgACC). Deactivations in these regions have frequently been reported in pain studies (Henderson et al. 2006; Kong, Loggia, et al. 2010; Porro et al. 1998) and are often hypothesized to result from the default mode network (Raichle et al. 2001) being more active in the control condition (Buckner et al. 2008). In our study, however, vmPFC and pgACC showed a specific behavior, as they were found to be involved in mediating the autonomic effect of acupuncture stimulation. Together with the dlPFC they were the only areas of those found for the pain-dimension of acupuncture that were still significant, when the results were weighted with the subjects' autonomic responses indicating their involvement in mediating the autonomic response to the acupuncture stimulus. While the vmPFC / pgACC showed decreased activity in subjects with a strong autonomic response, the dlPFC showed the opposite behavior.

A possible role of the dlPFC in autonomic regulation has been reported by several studies (Maihöfner et al. 2011; Napadow et al. 2008; Lane et al. 2009) and there is a substantial body of evidence for the vmPFC being a key cortical center of autonomic nervous system control (Verberne & Owens 1998; Wong et al. 2007). Activity in the vmPFC / pgACC has been reported to correlate
with autonomic nervous system activity measured by heart rate (Ziegler et al. 2009), skin conductance response (SCR) (Dubé et al. 2009), muscle sympathetic nerve activity (MSNA) (Macefield et al. 2006), cutaneous blood flow in glabrous skin indicating sympathetic vasoconstrictor activity (Maihöfner et al. 2011) and blood pressure (Sander et al. 2010). Furthermore, dlPFC and vmPFC are closely interconnected, as transcranial magnetic stimulation (TMS) of the dlPFC has been shown to strongly influence activity in the vmPFC and pgACC (Paus et al. 2001).

The brainstem showed a behavior similar to the cerebral cortex in that different activations were observed for the pain and the autonomic dimension of the acupuncture stimulation. The needling sensation correlated with increased activity in the locus coeruleus (LC). Again, this nucleus is known to play a central role in pain processing (Heinricher et al. 2009; Ossipov & Dussor 2010). The autonomic dimension of the acupuncture stimulation was found to be mediated by a mesencephalic and brainstem network comprising the hypothalamus, locus coeruleus, and the ventrolateral medulla (VLM). All of these areas have been shown by other groups to be involved in autonomic regulation (Thayer et al. 2009; Macefield et al. 2006; Macefield & Henderson 2010; Napadow et al. 2008). Experiments applying acupuncture stimulation in rodents and cats have identified a network consisting of the hypothalamic arcuate nucleus, the ventrolateral PAG and the rostral VLM to mediate cardiovascular effects of acupuncture (Li & Longhurst 2010). In detail, the acupuncture stimulation above the median nerve but also direct nerve stimulation (Li et al. 1998) were found to inhibit sympatho-exitatory signals of the rostral VLM (Tjen-A-Looi et al. 2006), an effect mediated by hypothalamic areas (Li et al. 2009).

The connection between cortical and subcortical activations observed in our study may lie in direct projections from the vmPFC / pgACC to hypothalamic nuclei like the arcuate nucleus. Such connections have recently been demonstrated for the PAG on human subjects by means of tractography and functional connectivity studies (Kong, Tu, et al. 2010; Hadjipavlou et al. 2006).
The PAG then projects to the VLM and inhibits sympa tho-exitatory signals (Li & Longhurst 2010). The suggested mechanism of acupuncture's autonomic effect is subsumed in Figure 5, which shows typical cortical and brainstem centers of pain and autonomic processing as well as their extensive interconnections. We hypothesize that acupuncture is processed by these centers and identified as a low-intensity deep pain stimulus, which then leads to a passive coping response mediated by the hypothalamic arcuate nucleus, the locus coeruleus and the ventrolateral medulla ultimately resulting in sympathoinhibition with observable effects like slowing of the heart rate.

Several studies have recently uncovered the cortical and subcortical networks involved in placebo analgesia (Eippert et al. 2009; Enck et al. 2008). In view of these studies and the ongoing debate if acupuncture is effective beyond the placebo effect (Ernst 2006), it may be interesting to note the striking similarities of the cortical and brainstem networks activated by both interventions. For example Eippert et al. reported activations in the dIPFC, rostral ACC, hypothalamus, PAG and RVM (Eippert et al. 2009), corresponding to exactly the same network of areas as observed in our study. This strong similarity may at least partly explain, why it has been so difficult in clinical studies on acupuncture to separate specific from unspecific effects.

Limitations of this study that must be addressed are the low spatial and temporal resolution of fMRI measurements and, subsequently, the inability of our method to resolve small anatomical details and fast changes in neuronal activity. Thus, the temporal interplay of the nociceptive and autonomic network nodes could not be revealed. Furthermore, the exact localization of the VLM activation and, thus, identification of this nucleus was hindered by the lack of probabilistic, stereotactic atlases for this part of the brain. Because the only available atlas of this kind (Afshar et al. 1978) that we used in our previous study (Beissner et al. 2011) contains only a limited number of nuclei, it was not suitable for the current one. Thus, we had to rely on a non-probabilistic, non-stereotactic atlas (Naidich et al. 2008) and base our identification of the nucleus on anatomical landmarks. Further limitations are set by the lack of control conditions in our study. Our claim that acupuncture is a
special deep pain stimulus is based on indirect observations (pain descriptors and autonomic reactions), while the proof of this interpretation would necessitate an actual comparison of the acupuncture stimulus to other well-characterized superficial and deep pain stimuli, like heat pain (cf. Kong et al. 2010) and injection of hypertonic saline (cf. Henderson et al. 2006). Furthermore, it is important to notice that this study does not allow any conclusions on the specificity of the chosen acupuncture point (Pc6) as the study did not involve a sham point control.

In summary, cortical and brainstem activations under acupuncture at acupoint Pc6 indicate that acupuncture may be a deep pain stimulus, which evokes a passive coping response leading to a reduction of sympathetic and a smaller increase of vagal activity. This autonomic concomitant of the pain stimulus is mediated by network of cortical, mesencephalic and brainstem areas and may explain acupuncture's non-analgesic effects and in some cases even have a therapeutic potential.

Acknowledgements

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References


Beissner, F., 2011. fMRI studies of acupuncture mechanisms - a critique. FACT, 16(1), 3-11.


Raichle, M.E. et al., 2001. A default mode of brain function. *Proc Natl Acad Sci USA*, 98(2), 676-


Table legends

**Table 1**: Correlation coefficients and associated p-values for acupuncture sensation time courses and those of heart rate, respiration rate and RMSSD (vagal component of heart rate variability). Mean p-values (starred) are the result of a one-sample t-test.

**Table 2**: Cortical and subcortical activations for the two statistical tests grouped according to cerebral and brainstem localization. First row ("Acupuncture sensation"): One-sample t-test on first-level results obtained by using needling sensation time courses as predictors. Second row ("Acupuncture sensation weighted by heart rate response"): Additional weighting of single subjects by their individual correlation coefficients from Tab. 1.

Figure legends

**Figure 1**: Experimental design of the study.

**Figure 2**: Individual time courses of the acupuncture needling sensation (black) assessed by repetitive rating on a visual analogue scale (VAS) and of the heart rate (blue) derived from subjects' pulse measurements. P-values are given for the correlation of both signals. For correlation coefficients see Tab. 1. Note: To ease visual comparison, hear rates are displayed with inverted scales.

**Figure 3**: Cortical BOLD responses to acupuncture at Pc6. The first row shows the results of a one-sample t-test on first-level results obtained by using needling sensation time courses as predictors. For the second row subjects were weighted by their individual correlation coefficients from Tab. 1. Of the areas that process the needling sensation (first row), only the ventromedial prefrontal cortex and the dorsolateral prefrontal cortex were involved in mediating the heart rate response (second row). The lateral occipital cortex / fusiform gyrus was not activated by needling sensation alone but
showed a deactivation in the weighted analysis. All data are presented in neurological convention. Abbreviations: SI / SII: primary / secondary somatosensory cortex, SFG / MFG / IFG: superior / medial / inferior frontal gyrus, LOC: lateral occipital cortex, ACC: middle anterior cingulate cortex, vmPFC: ventromedial prefrontal cortex, dLPFC: dorsolateral prefrontal cortex, pgACC: perigenual anterior cingulate cortex.

**Figure 4:** Brainstem BOLD responses to acupuncture at P6. The upper image shows a median sagittal section from the corpus callosum to the lower brainstem. Positions of transversal sections (a-c) are marked with their relative distance to the obex and are parallel to an imaginary line connecting anterior and posterior commissure. The center of the figure shows the results of two different second-level analyses (rows) superimposed on the anatomical image (columns). First row: One-sample t-test on first-level results obtained by using needling sensation time courses as predictors. Second row: The same test with subjects weighted by their individual correlation coefficients from Tab. 1. Third row: Respective transversal sections from a brainstem atlas (Naidich et al. 2009). All sections are presented in neurological convention. Circled activations / deactivations are the ones that reached statistical significance. Abbreviations: VLM: ventrolateral medulla, LC: locus coeruleus, Hyp: hypothalamus, Amy: amygdala.

**Figure 5:** Putative mechanism of acupuncture-induced heart rate changes based on this study and previous knowledge from animal research. Areas in blue font were those found activate in our study. Light blue indicates a role in processing of the pain dimension (needling sensation) but not of the autonomic dimension (heart rate response). Dark blue indicates involvement in the autonomic dimension or both dimensions. Connections between brainstem nuclei are largely based on Heinricher et al. 2009 and Ossipov et al. 2010. Abbreviations: vmPFC / dLPFC: ventromedial / dorsolateral prefrontal cortex, pgACC: perigenual anterior cingulate cortex, ACC: middle anterior cingulate cortex, SI / SII: primary / secondary somatosensory cortex, Hyp: hypothalamus, PAG:
### Cardiac-gated fMRI measurement

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<td>1 min</td>
<td>~10 min (depending on heart rate)</td>
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### During fMRI scan:

- Repetitive VAS rating of needling sensation intensity (approx. every 10 seconds)
- Covert feedback of VAS ratings below 20 points to the acupuncturist via headphones
- Needle stimulation with the aim to maintain VAS score > 20
- Continuous measurement of heart rate

### Result: individual time-courses of needling sensation

![Graph showing time-courses of needling sensation](image)

blocks of needle stimulation

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Fig. 1
Fig. 3
Fig. 4
Fig. 5
Table 1

<table>
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<th>respiration rate</th>
<th>RMSSD</th>
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<td>p</td>
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