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ORIGINAL ARTICLE

Difference of brain functional connectivity between regions of interest in patients with Osteoarthritis pain and opposed response to placebo

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Abstract

OBJECTIVES: This is a single subject clinical study to find out if there are any differences in functional connectivity between regions of interest in brains of patients with Osteoarthritis who respond to placebo (placebo responders) and those who do not (non-responders) to get a placebo predictor.

METHODS: The data gathered from a previous study was used. Two patients with different response to placebo and a healthy subject were selected for analysis. Echo planar imaging (EPI) and resting MRI were performed at the beginning of the trial. Pearson correlation and the distance correlation are two methods that have been used for checking the functional connectivity. Regions of interest were selected by Automated Anatomical Labelling 2 (AAL2).

RESULTS: A strong connection between right precentral gyrus, right and left superior frontal gyrus with left precentral gyrus had been found in the placebo responder patient as well as the healthy subject. The high relation existed between Cerebellum and vermis with the superior frontal medial, the anterior and the lateral orbital in placebo responder.

CONCLUSIONS: The placebo responder had the same brain connections as the healthy person. The most functional connectivity differences were the connections of Cerebellum and vermis with other regions in the responder that there were not in the non-responder. It can be concluded the existence of connections of this region with others can be used as a placebo predictor.

Introduction

Pain is one of the most encountered health problems that people struggle with these days and can be defined as acute or chronic. Acute pain has a distinctive source and does not last for more than 3 months however it is sharp and severe (Carr & Goudas 1999). On the other hand, chronic pain is a distinct pain that exist every day or most days, usually lasting more than two months, it should be noted that it usually does not have the same symptoms as acute pain (Treede *et al* 2015, Von Korff *et al* 2016). Almost 35% of the population are strug-

gling with chronic pain; this statistic means high costs of medical care and consequently loss of part of salary every month (Rice *et al* 2016; Simon 2012). There are some more common chronic pains, Osteoarthritis (OA) is one of them, which can lead to disability and loss of productivity (Sharma *et al* 2006). Chronic knee pain is due to inflammation or muscular weakness in one or both knees which is defined as long-standing ache. This kind of pain can disturb daily activities of life and cause disturbed sleeps. Therefore, researchers are trying to find different ways to improve lifestyle of patients by producing a new pain relief, rehabilitation therapy and equipment, etc. Between these researchers, a group is trying to figure out about differences in people's brain who are living with this pain.

Accordingly, the challenge of these researchers is about encoding any pain index in the brain. As mentioned before, this contest becomes more important when the topic is about chronic pain while it can affect different aspects of a patient's life (Gracely 2004; Malfliet *et al* 2017). Pain is the set of sensory, emotional, cognitive responses to active internal and external conditions (Malfliet *et al* 2017). This makes pain a subjective experience, which makes it difficult to research about it. Though, there are some researches that showed allocation of pain in brain can be realized by functional connectivity (Bastuji *et al* 2016; Garcia-Larrea & Bastuji 2018). Thus, functional magnetic resonance imaging (fMRI) as a non-invasive technique brain imaging is chosen.

One another phenomenon that researchers encountered is the placebo effect. The therapeutic effects of the placebos are shown in some chronic pains such as low back, arm, knee (Finniss *et al* 2010; Finniss & Benedetti 2005; Vachon-Presseau *et al* 2018). However, the reason for this response to placebo is still not fully understood. The most important assumption of this influence is about brain connectivity.

As a result, this article tries to figure out if there are any differences in the patients' brains who respond to placebo (placebo responders) and those who do not (non-responders). These patients had OA as a bold chronic pain and takin MRI was done before starting treatment. Hence the data which was gathered by a previous study by (Tétreault *et al* 2016) was used.

Material and method

Participation

For this study two patients with the same gender and age but different responses to placebo were chosen from the study conducted by Tétreault *et al* (2016). Their clinical trial research number was ClinicalTrials.gov NCT02903238. Based on their study (study 1) 17 patients with knee OA participated in their trial, at the end of the 2-week placebo treatment period, they have been asked to complete the visual analog scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index(WOMAC) scores (Tétreault *et al* 2016). The result of these two indexes classified patients in two groups: first, those who their knee pain decreased significantly (placebo responder), second, those who their knee pain remained still (placebo non-responder). Since the focus of this study is to find the correlation between brain regions in detail, a patient from placebo responder, another from non-responder group and a healthy subject with the same condition were chosen.

Brain scanning parameters

A high-resolution T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE) anatomical brain images were obtained as described in the main study (Baliki *et al* 2012; Tétreault *et al* 2016). Concisely, a 3T Siemens Trio whole-body scanner with echo planar imaging (EPI) capability using the standard radio-frequency head coil with the voxel size 1×1×1 mm, TR = 2,500 ms, in-plane matrix resolution = 256×256; slices = 160; and field of view = 256 mm. On the same day resting state fMRI images were collected. The scanner had the following specifications: multi-slice T2-weighted echo-planar images with repetition time TR = 2.5 s, voxel size 3×3×3 mm, number of slices = 40, and in-plane resolution = 64×64; the number of volumes was 300 (Tétreault *et al* 2016). All MRI data are available on openfmri.org.

Data preprocessing

The first level of data analysis is image preprocessing therefore the SPM12 (http://www.fil.ion.ucl.ac.uk/spm) software was used for this purpose. Briefly, the anatomical (T1) images help to improve the accuracy of the functional EPI images. The following standard protocol was applied for preprocessing, including reorientation, alignment, coregistration, segmentation, normalize and smoothing.

Head motion in resting state fMRI means there would be additional steps in preprocessing. For this purpose, the combination methods which was benefited by Geerligs *et al* (2016) was applied. The Wavelet Despike method was exerted for monitoring for the bias that was created by head subtle motion artifacts from fMRI data without the need for data scrubbing (Patel *et al* 2014). The main idea of this method is to detect non-stationary events which are created by movements and then despiking these from voxel time series by the help of chains of wavelet coefficients (Geerligs *et al* 2016; Patel *et al* 2014). Data is then extracted for each region based on the Automated Anatomical Labelling 2 (AAL2) atlas (Rolls *et al* 2015). The next step for reducing any noise confounding including head motion was applying a general linear model (GLM).

Statistical analysis

Functional connectivity aims correlation of the BOLD signals between regions of interest (ROIs) (Nazari *et al* 2018; Zhanget al 2015). Therefore, strength of the connectivity between regions have been be calculated with

some measures such as Pearson correlation, Mutual Information or distance correlation. Between these measures, distance correlation can be calculated in two ways: univariate or multivariate. The advantage of the multivariate distance correlation, compared to the Pearson correlation and the univariate distance correlation, it is that it can calculate based on the voxels where they are averaging the signals across all voxels in each region separately (Pannunzi *et al* 2017; Smith *et al* 2011).

First of all, linear correlation between two signals was computed by the Pearson correlation. At the second step, the distance correlation which is a measure that computes linear or non-linear dependency between two regions (Székely *et al* 2007), was processed. The advantage of the distance correlation, is that it is an unbiased indicator, while the difference of the number of the voxel in each region could cause bias (Székely & Rizzo 2013).

One point about the distance correlation that should be emphasized, is that a similarity measures between time-points not correlation ones. The distance correlation is always a positive value, so, there is not difference between negative or positive associations. More details on statistical formulas were given on Supplementary data.

Results

The findings for two male patients of similar ages (54) but with different responses to placebo and one healthy man was analyzed. The matrix of the regions correlation for these three subjects are shown in the Supplementary Figure 1. The number of the axis shows the code of the region based on AAL2 in SPM12. However, it did not have a good visualization. For having a better conception about functional connections, BrainNet Viewer package have been used (Xia *et al* 2013). It should be noted that for comparing the connectivity between two measures of correlations, the absolute value of Pearson correlation was considered. Moreover, correlations above 0.6 were plotted (van den Heuvel *et al* 2017) in order to keep the figures clean enough. Figure 1 showed the result of this package. As seen in this figure, there are some differences

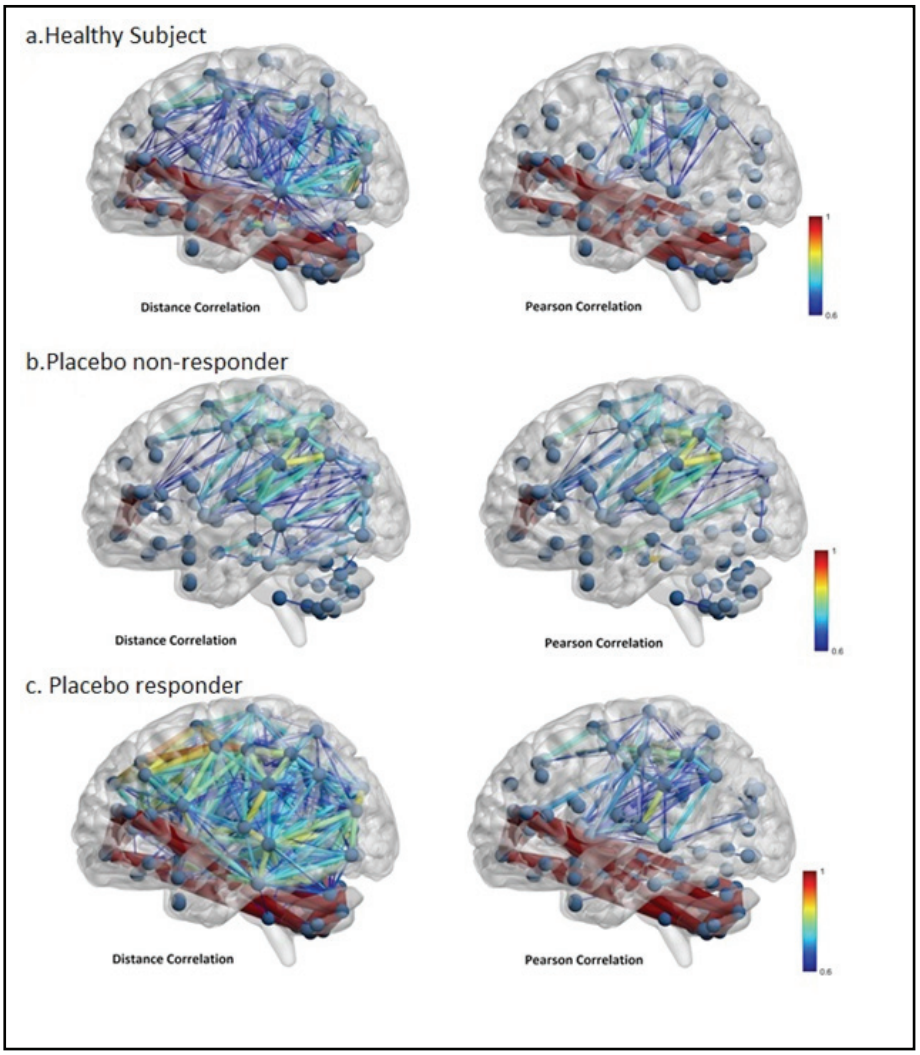

Fig. 1. Functional connectivity measured by distance and Pearson correlation in the healthy subject (a), the placebo non-responder patient (b) and the placebo responder patient (c).

in the connection between regions. There are some strong connections between regions in the patients' brains who responded to placebo that do not exist in the other patient. The distance correlation emphasizes these different connections in Pearson index.

The placebo responder patient had a strong connection between right precentral gyrus, right and left superior frontal gyrus with left precentral gyrus, as did the healthy subject. The other interesting connection that did not exist for the non-responder and also distance correlation confirmed it, was the high relation between Cerebellum and vermis with the superior frontal medial, the anterior and the lateral orbital. There were connections between supplementary motor area and Superior frontal in placebo responder which, did not existed even in healthy or placebo non responder. In AAL2 participations, Cerebellum and vermis were categorized in 25 parts together. As shown in Figure 1, more parts of the cerebellum and the vermis had connections with superior frontal medial, the anterior and the lateral orbital. On the other hand, one thing that can point to the distance correlation is that, connection between regions could be seen in placebo responder more than placebo non-responder and even the healthy participant. These connections exist mostly between the orbital surface, the subcortical grey nuclei and the Limbic lobe.

Discussion

As noted before, in this study whole brain scans were collected before treatment were used by patients. Therefore, if any changes exist in brain region links between subjects, it was not the cause of the placebo effect. The most significant result of this study is that patients who do not respond to placebo had less connection between brain regions than the healthy control and the placebo responder. And the other hand, there were some connections.

The previous study (Tétreault *et al* 2016), found the highest significant difference in connections between the right mid frontal gyrus and other parts in placebo responder compared to non-responder.

The most important difference between this study and the previous one (Tétreault *et al* 2016) was in region selection. The cerebellum and the vermis were two regions that had not been considered in the previous study, where in this paper we have been shown that placebo non-responder did not have connection between these two regions and some other regions. Moreover, this connection was seen to be strong in the placebo responder compared with the healthy control.

Conclusion

It can be concluded that connection between different parts of the cerebellum and the vermis with superior frontal medial, the anterior and the lateral orbital could be considering in the future research to be as a placebo predictive.

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Supplementary information is available in the online version of the paper.

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Supplementary data - Difference of brain functional connectivity between regions of interest in patients with Osteoarthritis pain and opposed response to placebo

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Suplementary Data.

If x and y was two signals with n time point, and where $\bar{x}$ and $\bar{y}$ is the mean of x and y respectively, therefore the Pearson correlation is:

$$Pcor_{xy} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

For defining the distance correlation(Székely, Rizzo, & Bakirov, 2007), let us suppose X and Y as two matrices of n time points by v voxels. Calculating Euclidean distance in voxel-space between each pair of time points is the first step. AS X and Y represent two separate regions, it should be computed in each matrix separately:

$$a_{i,j} = \sqrt{\sum_{k=1}^{v}(X_{ik} - X_{jk})^2} \qquad i,j = 1,\dots,n,$$

$$b_{i,j} = \sqrt{\sum_{k=1}^{v}(Y_{ik} - Y_{jk})^2} \qquad i,j = 1,\dots,n.$$

The main advantage of the distance correlation is that it is an unbiased predictor, for this purpose, U-centering is applying instead of double centering. The U-centering for matrix X is given by:

$$A_{i,j} = \begin{cases} a_{i,j} - \frac{1}{n-2}\sum_{l=1}^{n} a_{i,l} - \frac{1}{n-2}\sum_{k=1}^{n} a_{k,j} + \frac{1}{(n-1)(n-2)}\sum_{k,l=1}^{n} a_{k,l}, & i \neq j; \\ 0, & i = j. \end{cases}$$

After centering the distance matrices, the distance covariance computed by:

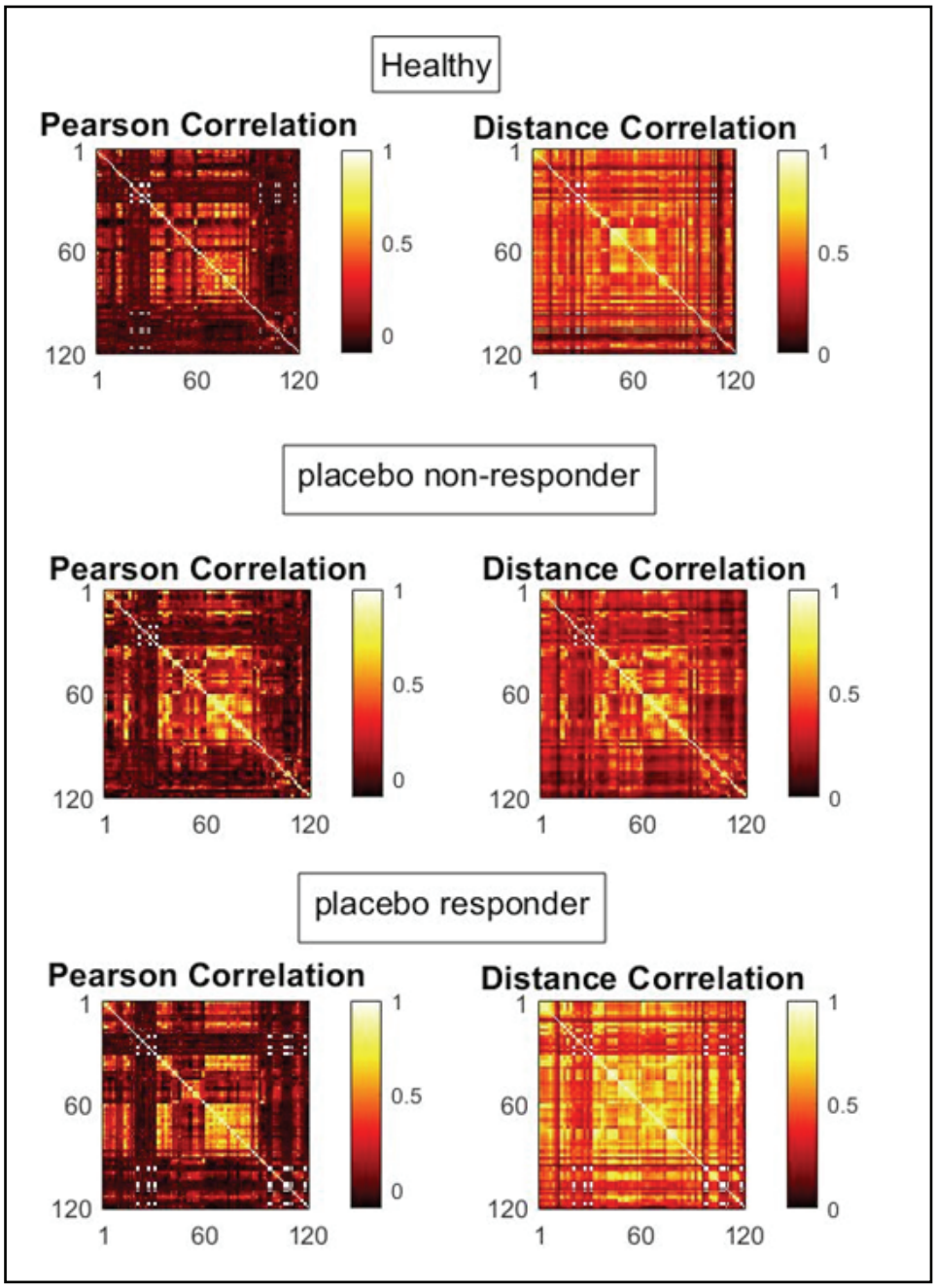
$$dCov(X,Y) = \frac{1}{W}\sum_{i,j=1}^{n} A_{i,j}B_{i,j}$$

And therefore the distance variance is written as follows:

$$dVar(X) = \frac{1}{W}\sum_{i,j=1}^{n} {A^2}_{i,j}$$

Element W is the normalization factor and in the U-centering is equal to n(n-3).
In conclusion, the distance correlation is specified by:

$$dCor(X,Y) = \begin{cases} \sqrt{\frac{dCov(X,Y)}{dVar(X)\,dVar(Y)}} & dCov(X,Y) > 0 \\ 0, & dCov(X,Y) \leq 0 \end{cases}$$



Suplementary Fig. 1. Functional connectivity matrices based on the set of AAL2 ROIs.

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