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Decoding acupuncture electrical signals in spinal dorsal root ganglion

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ABSTRACT

Neural system characterizes information in external stimulations by spatiotemporal encoding. In order to reveal the underlying mechanisms about the conduction and function of acupuncture signal, experiments are designed that is different types of manual acupuncture (MA) manipulations are taken at 'Zusanli' points of experiment rats, and the induced electrical signals in spinal dorsal root ganglion are detected and recorded. First, the firings of neuronal clusters are distinguished by extracting features of each spike shapes. Then types of acupuncture manipulations taken on the rats are inferred with a high probability by Bayesian decoding algorithm based on each single trial. Data in the first 200 ms from acupuncture onset are recognized to play a crucial role in increasing the decoding performance in all sessions. These results are proved to be significant by statistical analysis. These studies have offered new insights into neural processing underlying acupuncture and may help to construct the interface between neural systems and machines and improve the clinical study.
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1. Introduction

A primary task of neural system is the representation of environmental information [1]. Although single neuron recording is the standard tool in investigating the relationship between stimuli and responses in neural systems [2], the information carried by the single neuron is not sufficient to understand how neuronal populations process information. To further understand these mechanisms, multiple-neuron, single trial methodologies are proposed instead of single-neuron, multiple-trial framework, because neural system processes information based on each single event [3]. Information can be extracted from the neuronal population recording by spike-sorting and decoding algorithms [4–6]. The decoding of visual inputs such as orientation, spatial location, and images have been reported [3,7–9]. Furthermore, the framework of encoding and decoding through a bi-directional neural interface has been investigated [10]. However, whether acupuncture as a kind of external stimulus can be revealed from the perspective of encoding and decoding is still unknown.

Acupuncture is an essential part of traditional Chinese medicine (TCM) and it has been proved to be effective for the treatment of diseases [11–13], especially in the treatment of pain [14,15]. It has been demonstrated that the nervous system, neurotransmitters and endogenous substances respond to acupuncture [16–20]. The underlying molecular mechanisms of acupuncture effects have also been widely studied [21,22]. However, the underlying mechanism of acupuncture and whether acupuncture information is transmitted by electrical signals are still unclear.

Classical acupuncture literature holds that manual acupuncture (MA) with different stimulation types exert differential effects on body functions [23]. MA with different amplitudes and frequencies differentially modulate cerebral blood flow velocity, arterial blood pressure and heart rate in human subjects [24]. Clinically, there are three main types of MA which are differentiated by manipulations of the inserted acupuncture needle: 1, twisting; 2, lifting and thrusting; 3, a combination of 1 and 2 [25]. These three types of MA have different effects on blood pressure in the anesthetized rat [26]. However, why different types of MA have different effects are still unknown.

Many investigators focus on the studies of the electrical signals induced by acupuncture, e.g. electromyography and brain electrical activities based on PET or fMRI [32–35]. 'Zusanli' point is one of the most effective points in medical treatment by acupuncture. Acupuncture on 'Zusanli' point could modulate visceral activity only through spinal reflexes [27]. Therefore the functions of acupuncture could be investigated after eliminating the effects of senior central nervous system. The acupuncture afferent pathways and central sites have been identified in the anterolateral tract in the spinal cord [28,29]. Neural electrical signals evoked by different types of MA have been characterized by nonlinear data analysis [30,31]. However, the differences between signals evoked by different types of MA are not obvious. Moreover, the underlying biological meanings of the nonlinear data analysis are also

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not sufficient. Therefore, the essence of different MA can be explored from a new perspective of spatiotemporal encoding and decoding.

Firstly, we designed acupuncture animal experiments, and obtained the neural electrical signals in spinal dorsal root ganglion of the experiment rats evoked by acupuncture on 'Zusanli' point. Then we applied spike-sorting algorithms to investigate the characteristics of spatiotemporal coding of different types of MA. Moreover, decoding algorithms have also been applied to predict the acupuncture manipulations. Finally, the conclusion is given.

2. Design of acupuncture experiment

The flow chart of the experiment design and data analysis is shown in Fig. 1.

Healthy Sprague Dawley rats (190–210 g) anesthetized deeply by 20% ethyl carbamate (1.5 g/kg) are prepared for the experiments. After dissecting the rat to expose the lumbar nucleus, nerve tracts in L4 spinal root which is corresponding to transmission path of acupuncture signals are separated. Transmission properties of four basic types of MA ('nb','nx','tb','tx') are investigated. Here, 'nb' and 'nx' manipulations belong to twisting type while 'tb' and 'tx' manipulations belong to lifting and thrusting type. Each type of manipulation is taken at 'Zusanli' points of experimental rats for 90–100 times periodically within 2 min. This procedure is designed according to the timing when acupuncture is used in clinical treatments. Considering the adaption of neurons to the acupuncture stimulations, the data induced by the acupuncture of the first 10 times are eliminated. Manipulations are stopped taking for 5 min between different patterns of MA to eliminate the effects of the previous MA. To keep the precision of neural encoding, the acupuncture needle is kept in the skin for the whole process of the experiment. The data are detected by platinum electrodes and recorded by MP150 (BIOPAC), and the sampling frequency is 40 kHz. The data come

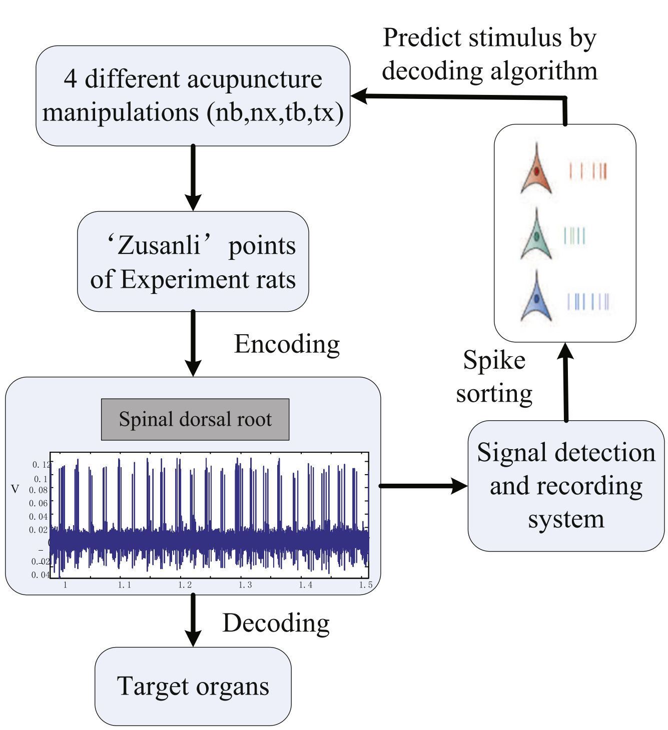

Fig. 1. Process of encoding and decoding of acupuncture.

from experiments of seven rats. One experiment on a rat with four types of MA taken is regarded as a session, and one time of acupuncture is regarded as a trial.

3. Results of spatiotemporal encoding analysis

3.1. Spike detection and sorting

The data recorded at spinal dorsal root ganglion contain firing information of multiple neurons. In the first step, the firing information carried by individual neurons could be obtained by applying spike-detecting and spike-sorting algorithm [4]. After band-filtering the data between 300 and 3000 Hz, we detect the spikes by setting the threshold at

$$Th = 4\sigma, \quad \sigma = median\left\{\frac{|x|}{0.6745}\right\} \tag{1}$$

where x is signal after filtering and σ is the estimate of the standard deviation of the noise. To diminish the interference of spikes, this estimation is based on the median value of the filtered signal [4]. The time series after band-filtering are shown in Fig. 2(a).

After the detection of spikes, the wavelet transform is used to calculate the features of the spike shapes, and 64 wavelet coefficients for each spike are obtained. Here, we implement a four-level multiresolution decomposition using Haar wavelets. Each wavelet coefficient characterizes the spike shapes at different scales. We aim to select 10 coefficients that best separate the different spike classes. The coefficients have a multimodal distribution. Given a data set x, the test compares the cumulative distribution function of the data $F(x)$ with that of a Gaussian distribution with the same mean and variance $G(x)$. Deviation from normality is then quantified by

$$\max(|F(x)-G(x)|) \tag{2}$$

Then we use superparamagnetic clustering(SPC) algorithm to assign spikes with similar shapes to one cluster. Superparamagnetic clustering is a stochastic method that does not assume any particular distribution of the clusters. These spike sorting algorithms are corresponding to Ref. [4], which has been proved to be effective in many applications [3,4,7].

After spike-detecting and spike-sorting, the spikes are assigned to three clusters based on their spike shapes as shown in Fig. 2(c). Different types of spike shapes are classified into different areas according to their 10 wavelet coefficients. There are three separated areas in the plane of two wavelet coefficients as shown in Fig. 2(b). Therefore the spike shapes can be classified into three clusters. No obvious firing can be observed if no acupuncture is taken. The spikes induced by different stimuli can be differentiated according to the inter-spike interval (ISI) time series of cluster 1 neuron.

3.2. Spatiotemporal encoding analysis of information carried by acupuncture signal

Differences between time-dependent firing rates evoked by different acupuncture stimuli are investigated. Raster plots of the three clusters in each trial are shown in Fig. 3(a)–(c). The onset times of the acupuncture cannot be accurately recorded in millisecond scale due to the manual acupuncture operations. To avoid these errors, the first spike time of cluster 1 neuron in each trial is considered as the acupuncture onset time. Because the spikes of cluster 1 neuron exist in all types of MA and are proved to be efficient to separate data of different trials. The horizontal axis

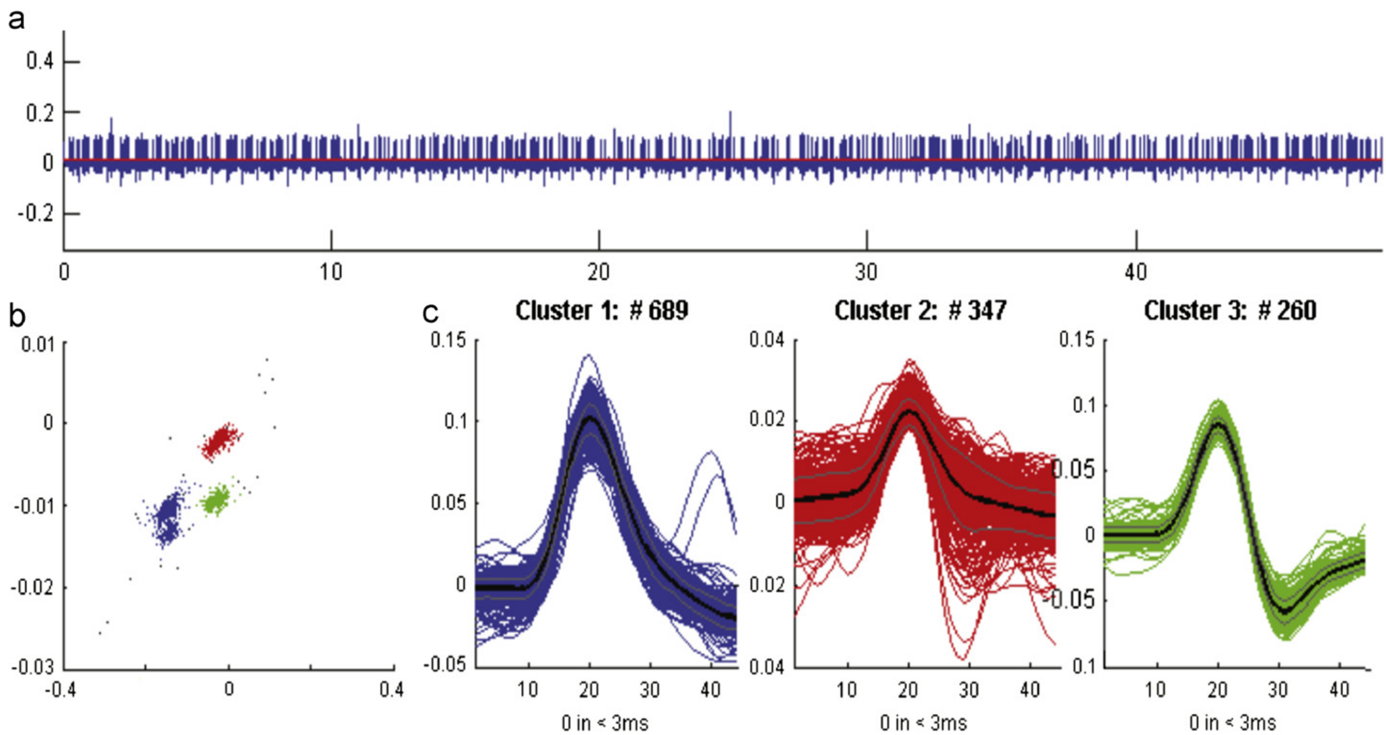

Fig. 2. Spike-sorting of signals detected in spinal dorsal root ganglion. (a) The signals after band-filtering between 300 and 3000 Hz; (b) distribution of wavelet coefficients; (c) spike shapes of different neuronal clusters.

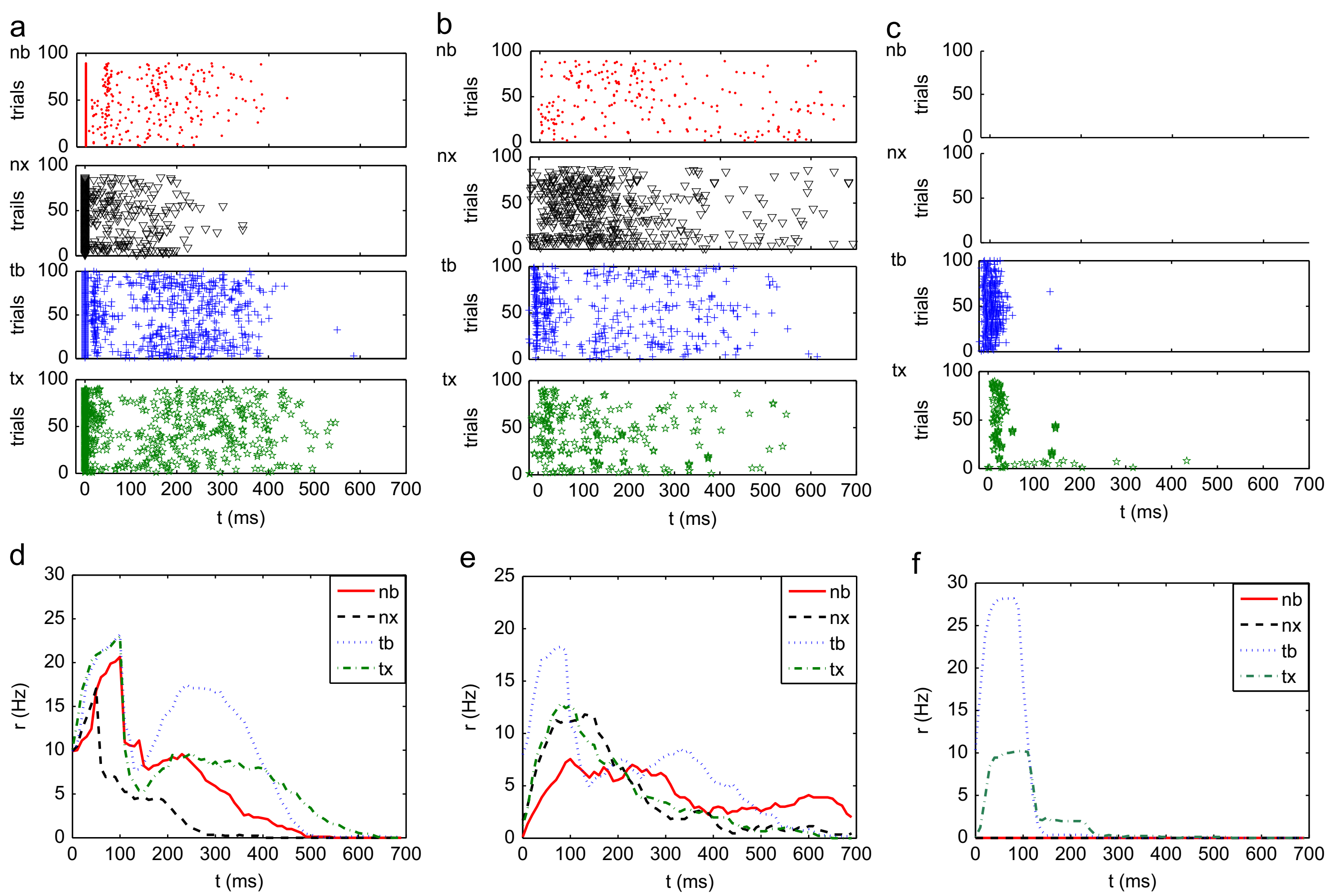

Fig. 3. Comparison of time-dependent firing rate of three clusters of neurons evoked by four types of MA. (a) Raster plots of cluster 1 neuron for all trials of a fixed MA. Four subplots represent results of 'nb', 'nx', 'tb', and 'tx' MA respectively; (b) Raster plots of cluster 2 neurons; (c) Raster plots of cluster 3 neurons; (d) PSTHs of cluster 1 neuron evoked by four types of MA; (e) PSTHs of cluster 2 neurons evoked by four types of MA; (f) PSTHs of cluster 3 neurons evoked by four types of MA.

denotes the time in one trial and the vertical axis denotes different trials with identical acupuncture method taken. Each point in this figure represents one spike. There is a cluster 1 neuron firing at $t=0$ ms in all the trials. However, for cluster 2 and cluster 3 neurons, their firings may be a little earlier than that of cluster 1 neuron, so spikes may occur at $t<0$ ms. It is shown that different acupuncture manipulations evoke different types of multiple neurons firings. Notably, firings of cluster 3 only exist when 'tb' and 'tx' MA(lifting and thrusting type) are taken as shown in Fig. 3(c).

PSTHs (Peri-Stimulus-Time Histogram) of the three clusters are shown in Fig. 3(d)–(f) respectively. The firing rates in a sliding time window of 100 ms are calculated. It is found that the encoding mechanisms of 'nb' and 'nx' manipulation are similar while those of 'tb' and 'tx' methods are also similar. However, the differences between PSTHs of 'nb' 'nx' and 'tb' 'tx' are obvious. These results are in accordance with the traditional classification of MA.

4. Extracting information from acupuncture signals

4.1. Predicting types of MA by decoding algorithm

Information transmission in nervous system involves the activity of neuronal populations. The signals evoked by different types of MA have their own features. However, the previous study is based on the average results of multiple trials, whether these manipulations can be distinguished from each other according to their responses in each single trial is still unknown. This problem determines whether different manipulations have different responses. Here, the problems are investigated in the perspective of neural decoding. Neural decoding, which refers to a reverse map to neural encoding, means reconstructing stimulus from the spike sequences it evokes. Here, we apply the neural decoding procedure according to the algorithms in Ref. [6] to our studies. Bayesian classification algorithm is applied to predict the MA taken on 'Zusanli' point by data recorded in single trial. It relies on the estimates of the conditional probability distribution of the neuronal responses for each stimulus from a given stimulus set. The Bayesian equation is

$$p(s|r)=\frac{p(r|s)p(s)}{p(r)} \tag{3}$$

s is one type of stimulus in all possible stimuli set $S=\{s_1,s_2\ldots s_n\}$, and r is one response in all possible responses set $R=\{r_1,r_2\ldots r_m\}$. In our study, S denotes the four kinds of manual acupuncture, and R denotes the possible number of spikes of the certain cluster of neurons.

$p(s)$ represents the probability of external stimulus s. $p(r|s)$ denotes the conditional probability of the r, given s. So the transmission path of signals evoked by acupuncture could be considered as a black box after ignoring the biological details. The transmission and function of acupuncture can be investigated by focusing on the relationship between stimuli and responses. Here stimuli are MA with different patterns, and responses are firings of multiple neurons in spinal dorsal root ganglion.

As an approximation, we may assume that the variability (for example, intrinsic noise) of the responses of the neurons to a given stimulus is independent. Note that this assumption does not imply independence of responses across stimuli [6]. So

$$p(r_1,r_2\ldots r_n|s)=p(r_1|s)p(r_2|s)\ldots p(r_n|s) \tag{4}$$

The Bayesian equation in the case of multiple neurons with equal probable stimuli can be written in the following form:

$$p(s|r_1,r_2\ldots r_n)=\frac{p(r_1|s)p(r_2|s)\ldots p(r_n|s)}{\sum_{l=1}^{n}p(r_1|s_l)p(r_2|s_l)\ldots p(r_n|s_l)} \tag{5}$$

The MA types are predicted based on the responses in one trial by Bayesian decoding algorithm. To evaluate to what extent different MA can be distinguished from each other. The decoding results are plotted in form of decoding matrix as shown in Fig. 4(a). The horizontal axis denotes the actual taken MA and the vertical axis denotes the predicted MA in the decoding matrix. The value on a given row i and column j in the matrix denotes the probability that the MA i is predicted to be MA j by decoding the responses. If there are no errors in the prediction, the elements on one diagonal line ($i=j$) are equal to 1, and the rest are 0. It means the

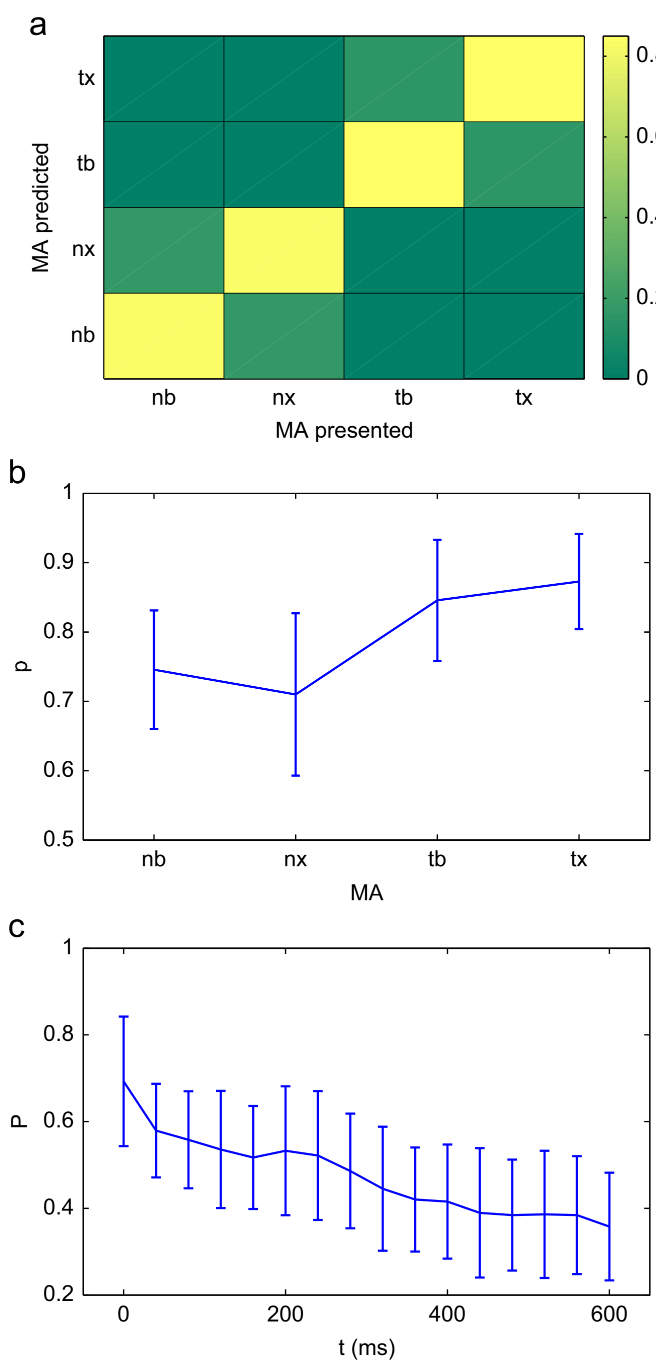

Fig. 4. Decoding of the signals evoked by acupuncture. (a) Decoding matrix of the signals in one experiment session; (b) probability of correct prediction (p) of four types of MA in all sessions. Error bars show 95% confidential interval; (c) comparison of decoding performance (P) of acupuncture information in sliding time windows (100 ms) of all trials in all sessions. Error bars show 95% confidential interval.

MA can be predicted only by the responses in one trial without any errors. All elements in the matrix are represented by different colors. The results on main diagonal lines of the decoding matrix in all sessions are shown in Fig. 4(b). It is found that the MA taken on the rats can be inferred with a high probability by decoding algorithm. Furthermore, MA 'tb' and 'tx' are easy to be predicted than MA 'nb' and 'nx'.

4.2. Statistical analysis

To evaluate the statistical significance of the decoding results, whether the percentage of correct prediction is larger than chance is tested by applying t-test. Then we assess the significance of decoding performances for each session. If the MA is predicted by chance, the predictions of acupuncture manipulations can be regarded as Bernoulli trials. The probability of correct prediction of each trial follows the binomial distribution. Given a probability p of correct prediction by chance ($p=1/M$, where M is the times of the trials). The probability of obtaining k correct predictions by chance in n trials is

$$P(k)=\binom{n}{k}p^k(1-p)^{n-k} \tag{6}$$

where

$$\binom{n}{k}=\frac{n!}{(n-k)!k!} \tag{7}$$

So we can calculate a p-value: $p\text{-value}=\sum_{j=k}^{n}P(j)$. Because p-values are less than 10^{-23} in all sessions, the decoding results in our study are significant.

4.3. Information analysis

Mutual information is the reduction of uncertainty about the stimuli after knowing the firings of multiple neurons. It is defined as

$$I(r,s)=\sum_{r,s}p(r,s)\log_2\frac{p(r,s)}{p(r)p(s)} \tag{8}$$

Here s is stimulus, r is response and $p(r,s)$ is the joint distribution. In this case, we quantify the decoding performances by applying the mutual information between the actual MA and the predicted MA. After calculating the data in all sessions, we obtain the range of mutual information is 1.48–1.87 bits with the average value 1.67 bits. As the information contained in the stimuli is 2 bits, This means most information in the stimuli can be recovered from the neuronal firings.

4.4. Time dependence of decoding

The previous decoding algorithm is based on the spike count during the whole process of acupuncture without considering the temporal factors. However, which period contains the crucial information that can differentiate MA has not been investigated. Here the mean values of the elements on the diagonal line ($i=j$) are used to quantify the decoding performance. Results of decoding in a sliding 100 ms time window are shown in Fig. 4(c). It is found that the decoding performance decreases with the sliding of the time window. The neural activities in the first 200 ms play a crucial role in differentiating the features of different MA. These results are in accordance with the time-dependent encoding results as shown in Fig. 3.

5. Conclusion

The present study shows that the encoding of different acupuncture manipulations have their own features. Different manipulations can be distinguished from each other with a high probability by decoding algorithm even in a single trial. These results imply that different coding features are related to the different effects which different acupuncture manipulations have. Considering the temporal factor in decoding, the data in the first 200 ms are found to play a crucial role in differentiating different manipulations. These results may help to construct neural interface for clinical treatment with acupuncture.

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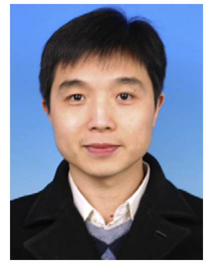

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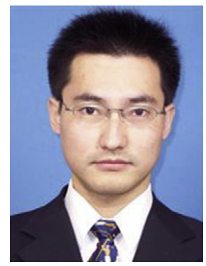

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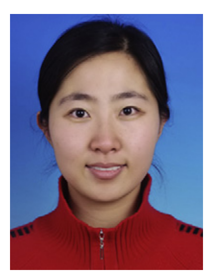

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