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Pig-Neural recording and analysis-workflow

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Abstract

Neural analysis using linear micrarray electrodes.

Troubleshooting

1 Recording and Raw Data

Neural data: 16 neural channels from 16 electrodes on the linear microelectrode array (LMA) were recorded. Electrode 1 is located at the tip of the LMA and it is linearly distributed until electrode 16. Each recorded channel is amplified and converted from analog to digital using CED system and then assigned a channel (ICN1, ICN2, ..., ICN16) in the Spike2 acquisition software. The recording from electrode i, is assigned to ICNi in the data file. The connecting wires of the multichannel electrode, along with earth and reference wires, were attached to a 16-channel microelectrode amplifier with headstage preamplifier (A-M systems, Inc., model 3600; Carlsborg, WA, USA). For each channel, filters were set to 300 Hz to 3 kHz, and gain to 5K.
RAE: A hook electrode was sewn to the atrial myocardium to provide a reference right atrial electrogram. This atrial electrogram was utilized for determination of atrial rate, duration. EGG is standard lead II electrocardiogram.

LVP: Left ventricular chamber pressure was measured via a 5-Fr Mikro-Tip pressure transducer catheter (Millar Instruments, Houston, TX, USA) inserted into the chamber via the left femoral artery.

2 Artifact removal

Artifacts were defined as a similar and simultaneous wave on all 16 channels. The source of artifacts might be from the electrical activity of the (atrium vs ventricle), electrical stimulation, motion artifact or might be unknown.
The following methods have been used to identify and remove the artifacts:
1- Identifying stim and heart electrical artifact: Electrical stimulation artifact were identified using the grass channel which is reflecting the exact time and shape of the stimulation. Heart electrical-related artifacts identified from onset detections of atrial and ventricular activities. An event channel was created based on the identification of the artifact and artifacts were removed by blanking the signal.
2-To identify the unknown source artifacts (in case they exist), a channel with no neural activity was selected and the event channel was created based on this identification. This waveform is present on all channels with similar morphology. Unknown source artifacts were removed by blanking the signal based on the event channel.

3 Signal to noise ratio

The amplitude of the extracellular action potential was divided by the maximum amplitude of the noise to calculate the SNR. Acceptable minimum SNR for analysis purpose was 3.

4 Hemodynamics

Heart rate (HR) was derived from the ECG signal. Thresholding was used to detect each beat and the HR curve was illustrated as instant frequency of detected events. The HR was shown in beat per minute. Dp/dt signal were created by taking the first derivative of the LVP signal. End systolic LVP curve was created by detecting the maximum value of LVP at the end of systolic phase. Dp/dt min/max was created using the maximum/ minimum value of the dp/dt channel

5 Spike Sorting

Detected spikes based on acceptable SNR criteria were identified. Identified spikes were classified using principal component analysis and cluster on measurement analysis. This classification assigned a label to each individual neuron.

6 High level Analysis

Sorted spikes and hemodynamic channels were transferred to Matlab software for higher level analysis.

-**Event triggered averaging**: Phase locked neural activity was assessed to find the correlation between the firing activity and cardiac cycle or electrical stimulation to investigate if specific cardiac phase or electrical stim triggers the spikes. Panel B shows example of unit activity relative to left ventricular pressure. Similar analysis can be used to ID neurons that are activated by autonomic stimulation: in this case the temporal firing pattern of units is summed based from delivery of stimulation to nerve.

-**Significant changes in the firing rate**:" Changes identified in neuronal activity were compared at different time windows by calculating the average firing rate over time. The significance level of the observed differences in firing rate was assessed using a statistical test recently utilized in a study of primary motor cortex neurons (Shin et al. 2010). The resulting P value is a function of four parameters: the duration of the two time windows and the number of firings in each time window.

The null hypothesis is that the two firing rates are equal. For this analysis, it is assumed that the number of action potentials identified follows a Poisson distribution in each time window and that the difference in the activities follows a Skellam distribution (Skellam, 1946; Strackee & Deneir van der Gon, 1962). Parameters can then be estimated using the maximum likelihood approach. From the Skellam cumulative distribution function, the probability that the difference in number of firings is larger than the observed value provides the desired P value (unilateral test). The test was implemented in Matlab and adapted from the R package 'skellam' by Jerry W. Lewis. Two significance levels were used: 1% and 5%.

The advantages of this method are its simplicity, the robustness of its parameter estimation and its applicability in the case of low firing rates, including when the firing rate is zero. Its limitations are the assumptions of stationarity and Poisson-distributed firings. In contrast with cortical neurons, neurons in the VIV ganglionated plexus tend to fire at low frequency unless a special event (e.g. ischemia) occurs. As a result, the limited number of firings in the time windows prevents robust estimation of a larger number of parameters. "(Beaumont,2013)

Using this approach, we utilized a battery of different stressors to assess functional responses of the peripheral autonomic ganglia. This included touching the right (RV) and left (LV) ventricular surface, transient great vessel occlusion of the inferior vena cava (IVC), aorta or bilateral carotid arteries (BCA) or electrical stimulation of the cervical vagi (right or left; RCV,LCV) or stellate ganglia (right or left; RSG, LSG). Significant increases in activity are indicated below as green; significant decreases in activity are indicated below as red. Note the diversity of the evoked responses indicative of the network processing occurring within this intrinsic cardiac ganglia.

-**Network analysis**: Network analysis was done using conditional probability. This type of analysis quantifies whether a neuron that responded to one stressor also responds to another stressor. For that purpose, a neuron was said to respond to a stressor when a significant change in its activity rate ($P < 0.05$; either an increase or a decrease) was observed before and during each intervention. The potential for a functional relationship between stressors X and Y was quantified within neurons identified in each subject as a conditional probability that a neuron that responded to stressor Y also responded to a stressor X. The conditional probability (Prob: response to Y | response to X) was estimated as the number of neurons that responded to both X and Y, divided by the number of neurons that responded to X.