

## **Analysis of cardiac reversal**

Heart videos were recorded at 30 fps. Then datasets from the tracking of the nuclei were first regularized by subtracting mean and dividing by standard deviation. Then data were resampled to 60.03 fps by interpolation of data points. That means that we calculated a value between each two data points creating a signal at 60.03 fps.

Then, we first identify peaks and troughs in the displacement of both nuclei. For a cell to be considered as beating first its peak/trough had to be 1 to 7 frames ahead of the peak/trough of the second cell. When the anterior cell peaked/troughed first at least 4 times in a row we classified it as a bout in backward direction. Conversely, when the posterior cell peaked/troughed first at least 4 times in a row we classified it as a bout in forward direction. Simultaneous peaks/troughs correspond to those where both cardiomyocytes showed maximal displacement in the same frame. These were infrequent, corresponding on average to 10% of the recorded peaks/troughs, and always isolated.

Having all anterograde, retrograde and simultaneous peaks/troughs identified, we next defined bouts of pumping either in the backward or forward direction, and points of reversal. For a sequence of beats in a particular direction to be considered a bout, at least 2 complete consecutive beats (a sequence of 2 peaks and two troughs) in the same direction were required. If a long sequence of beats in one direction, for example a forward bout, was interrupted by  $< 2$  consecutive beats (at most one peak and 2 troughs or two peaks and one trough) in the opposite direction, we considered the whole sequence as an uninterrupted bout, in this example a single forward bout. If, however, the interruption consisted of more than 2 beats (a sequence containing 2 peaks and 2 troughs) in the same direction, then we considered the pumping direction changed counting as a cardiac reversal. To determine the timepoints of cardiac reversal, we relied on the beginning and end of the backward pumping bouts as cardiac beating in these were more regular than in the forward bouts.

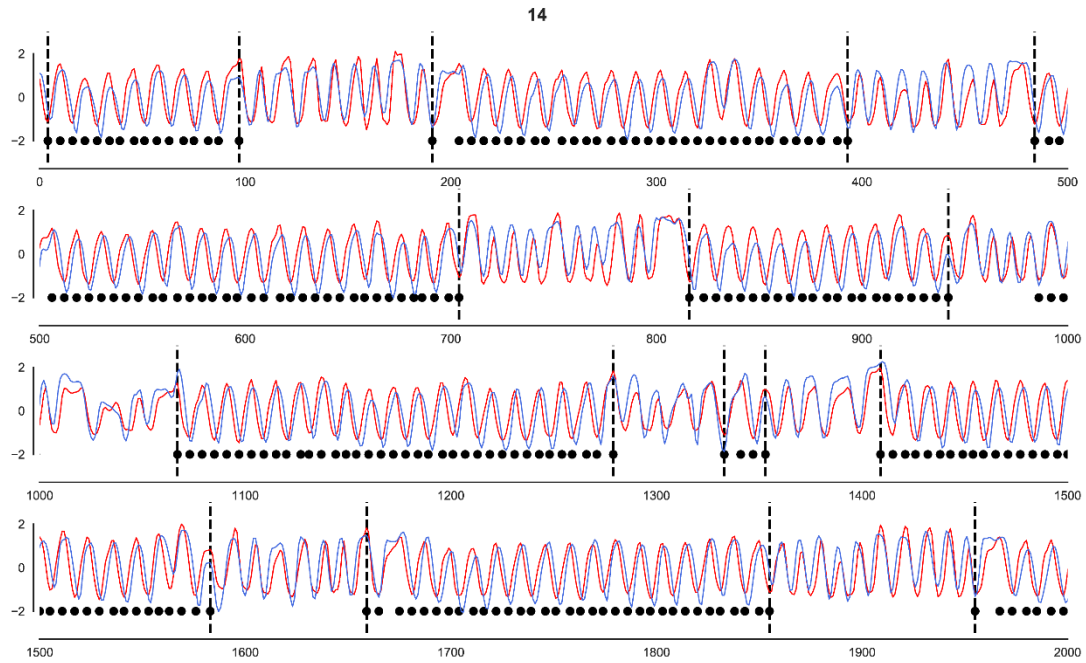
## **Selected examples of raw traces**

In each figure the displacement of the anterior and posterior nuclei is showed in red and blue respectively. Vertical dashed lines represent identified points of cardiac reversal. Black dots indicate identified backward peaks/troughs.

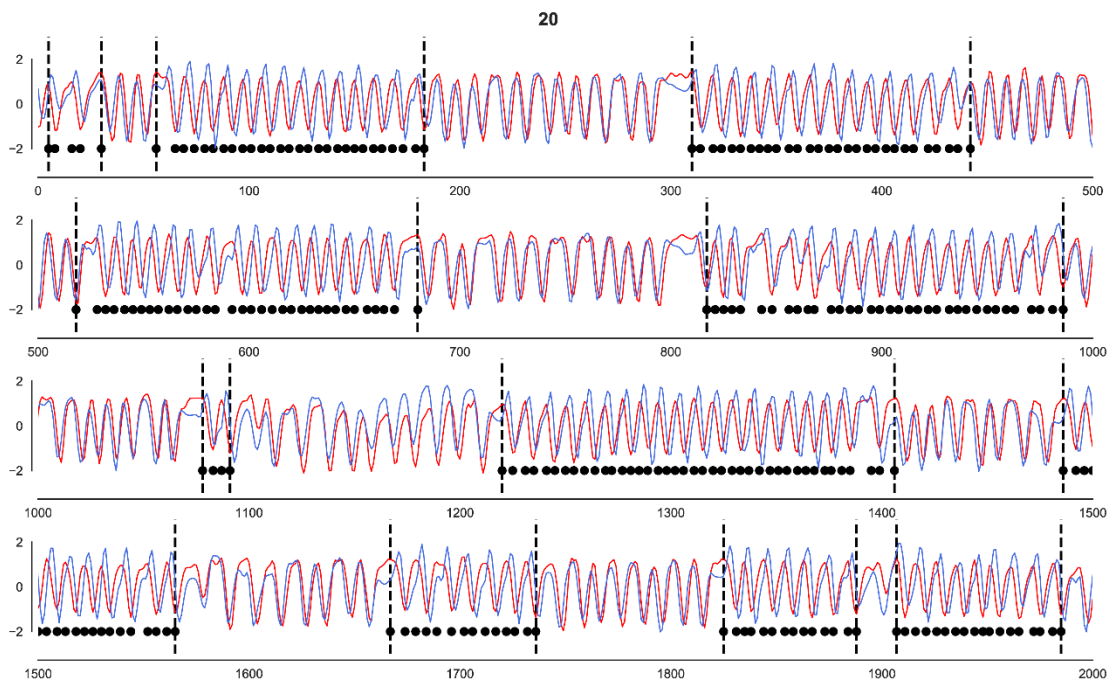
For each animal we have plotted the first 2000 frames (33,3 sec) of the baseline period .

1) Examples of traces of animals included

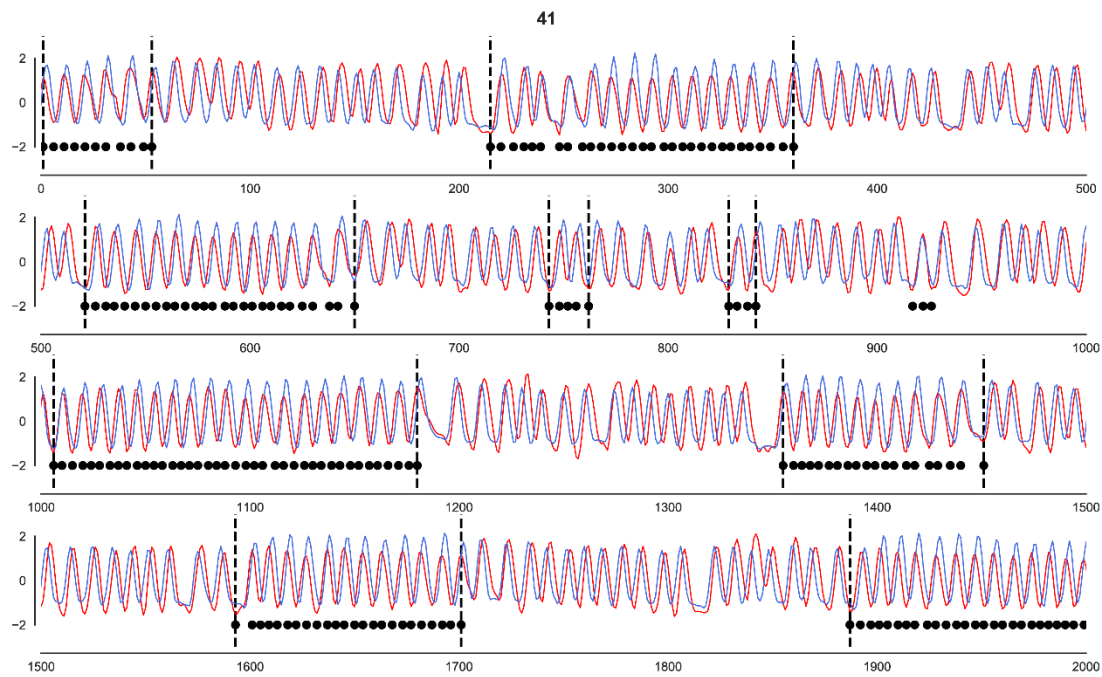
Animal 1\_14



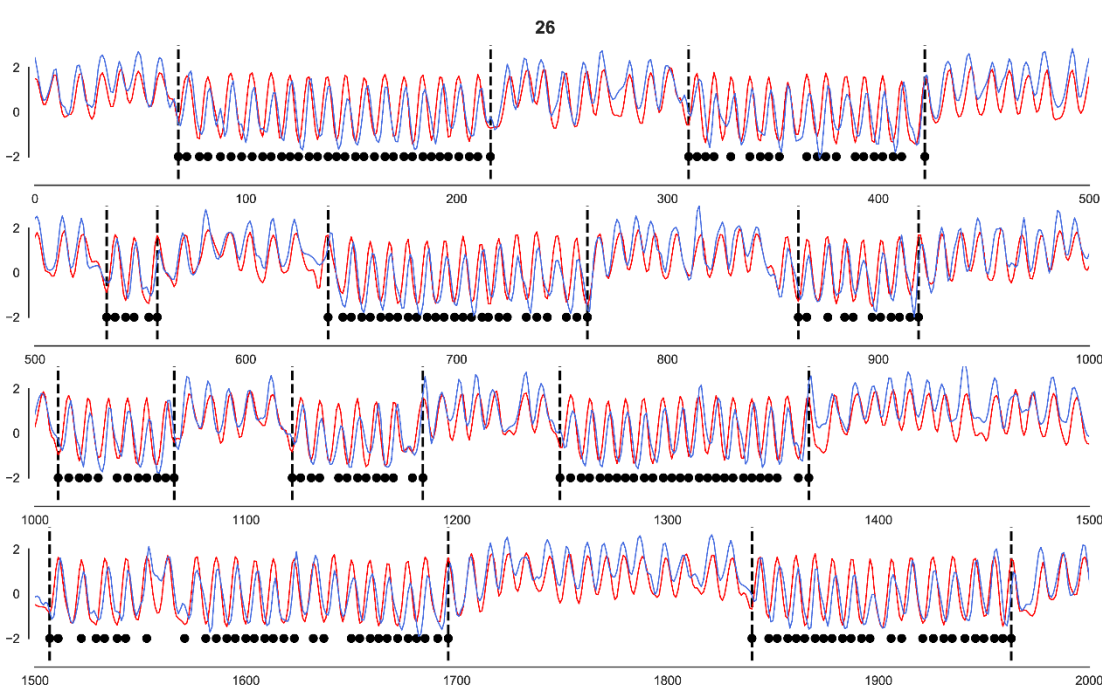
Animal 1\_20



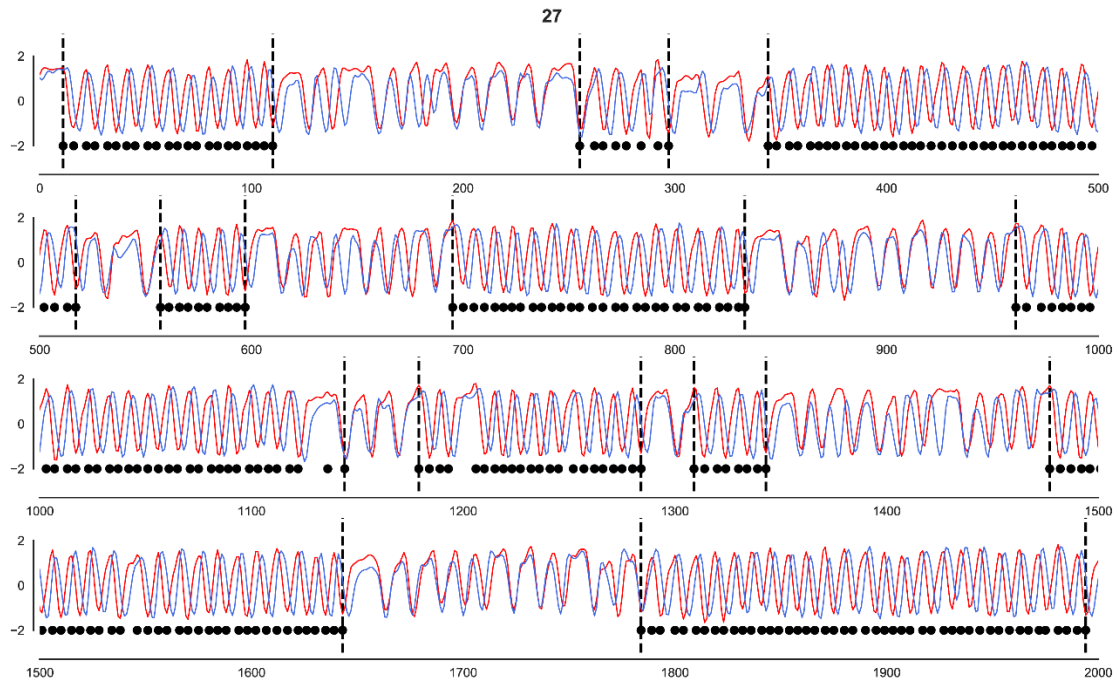
Animal 1\_41



Animal 2\_26



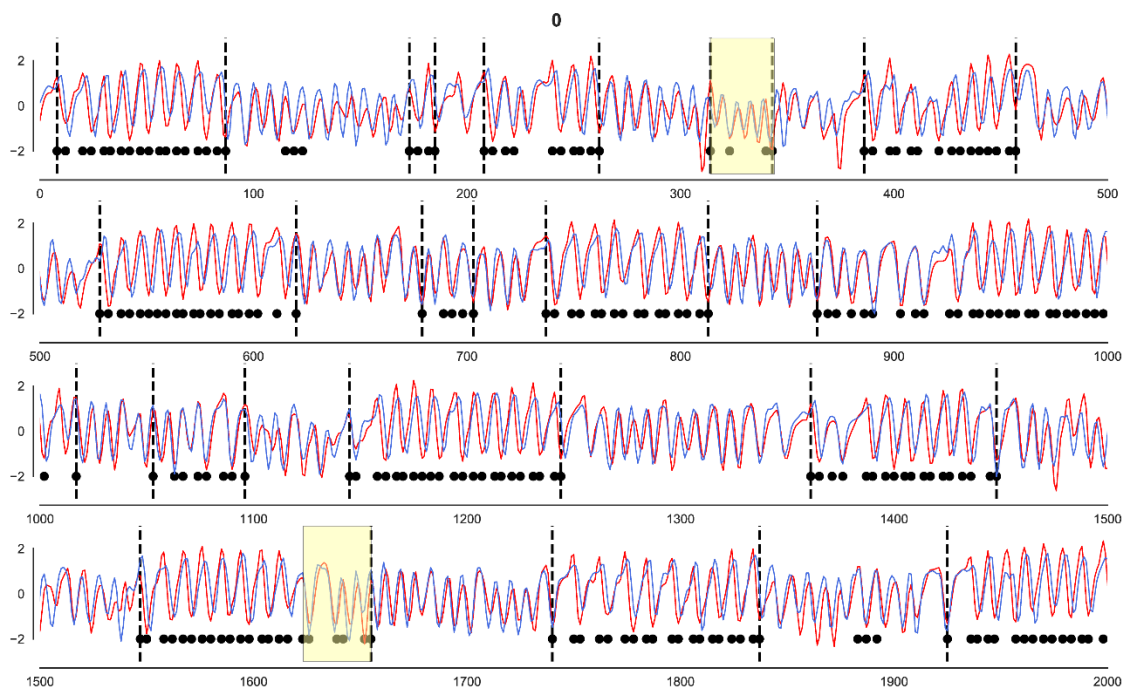
## Animal 2\_27



2) Example of errors in traces of included animals.

## Animal 1\_0

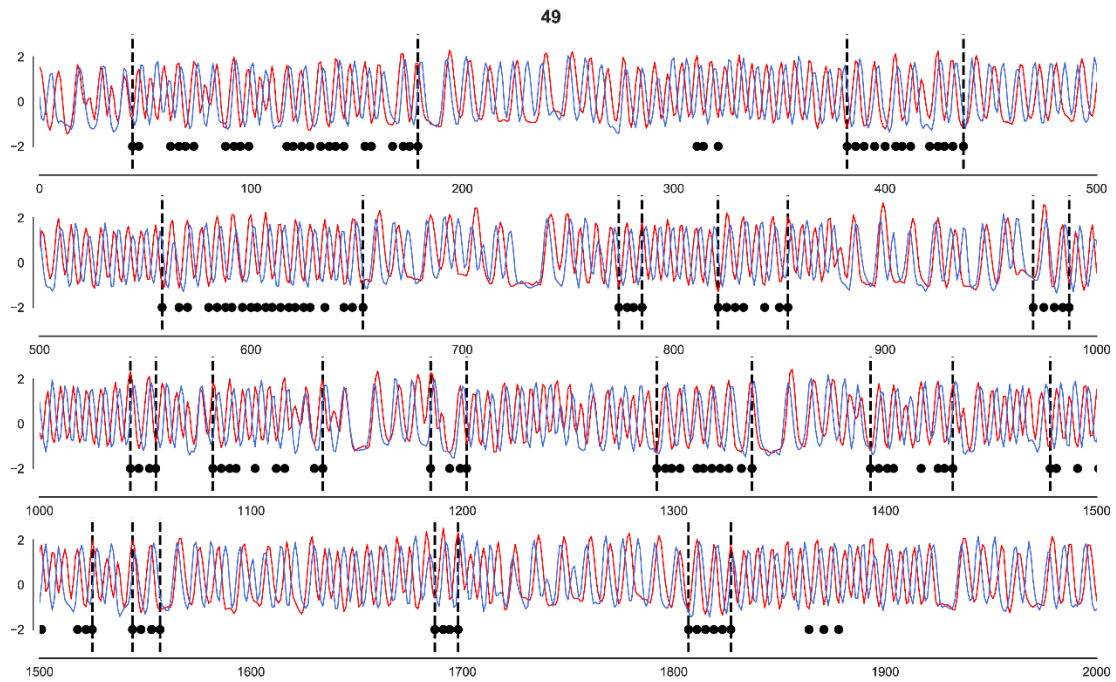
In this case, a small fraction of forward beatings are included as backward pumping (yellow).



### 3) Examples of traces of other excluded animals.

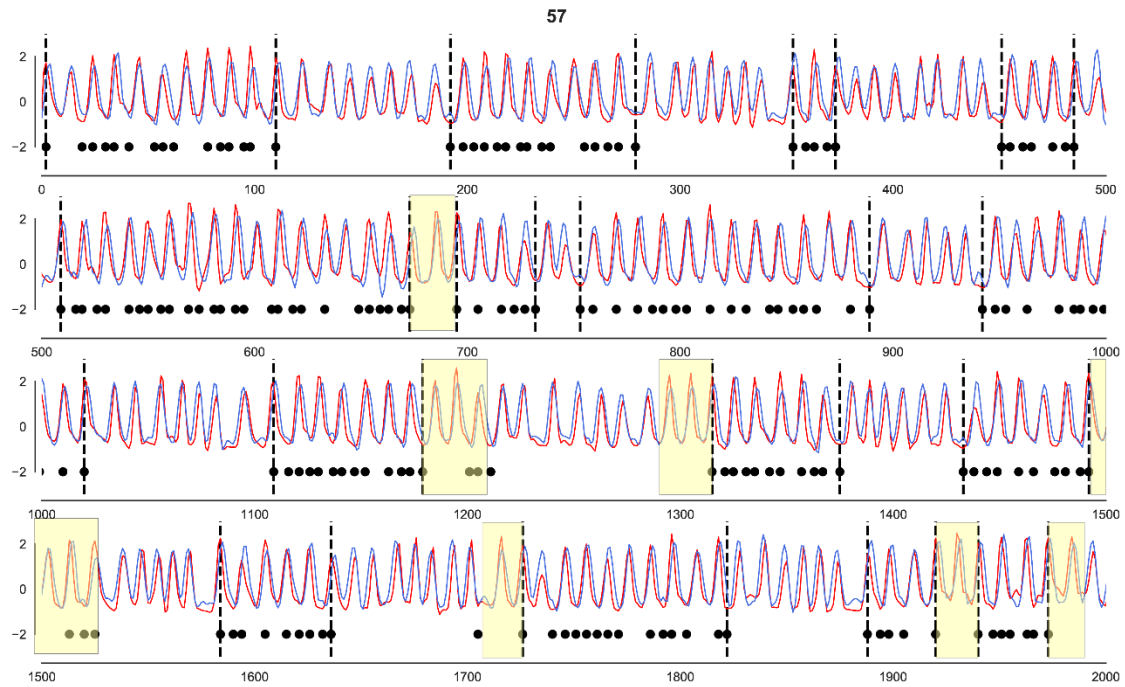
#### Animal 2\_49

This animal was excluded because anterior and posterior peaks/troughs are too separated and it was not possible to automatically differentiate backward from forward pumping bouts.



Animal 2\_57

Animal excluded because a fraction of backward beatings are included as forward pumping (yellow).

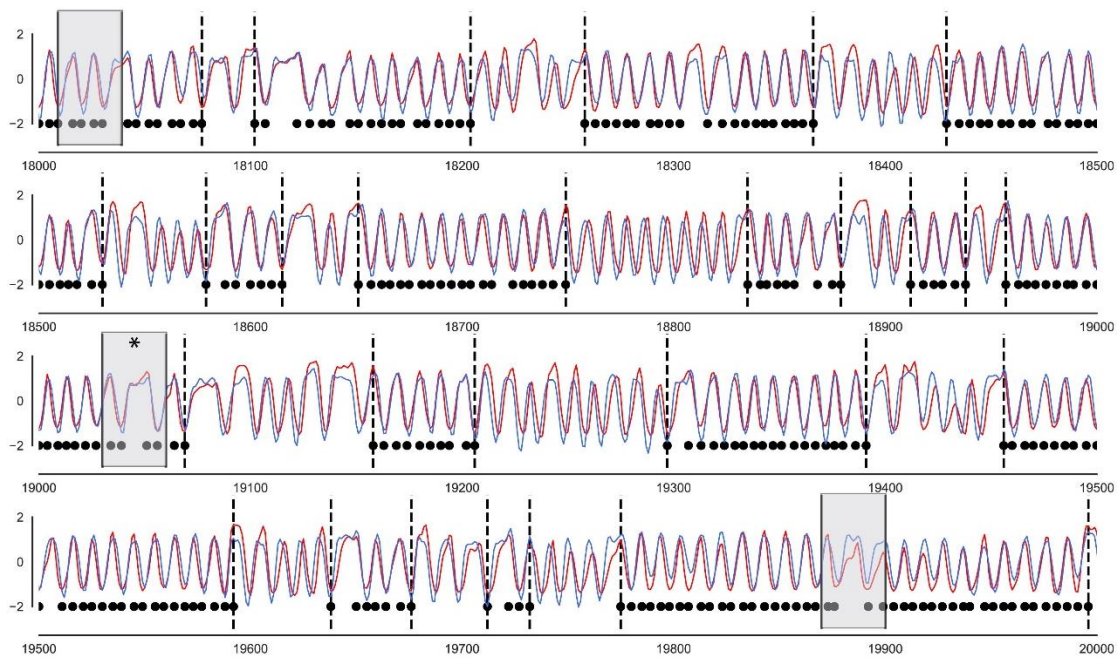


4) Examples of traces of included animals during stimulation.

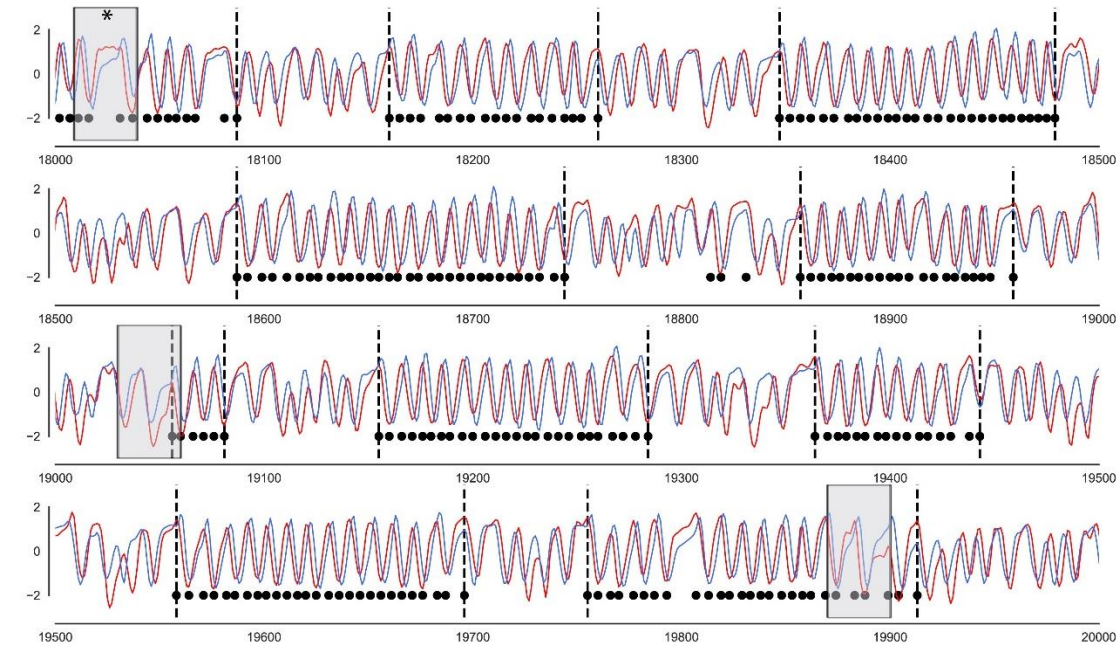
For each animal we have plotted the first 2000 frames (33,3 sec) of the stimulation period. In each figure looming presentation is shown as grey rectangles. The asterisks indicate identified cardiac arrest associated to looming.



Animal 1\_17



Animal 1\_8



Animal 1\_26

