Abstract—The precise identification of loss of consciousness (LOC) is key to studying the effects of anesthetic drugs in neural systems. The standard behavioral assay for identifying LOC in rodents is the Loss of Righting Reflex (LORR), assessed by placing the animal in the supine position every minute until it fails to right itself. However, this assay cannot be used when the rodents are head-fixed, which limits the use of powerful techniques such as multi-electrode recordings, in-vivo patch clamp, and neuronal imaging. In these situations, an alternative way to assess LOC is needed. We propose that loss of movement (LOM) in whiskers and paws of head-fixed animals can be used as an alternative behavioral assay in head-fixed animals. Unlike LORR, LOM in whiskers and paws is much harder to detect by visual inspection. Therefore, we developed a method to automatically assess for LOM of whiskers and paws in head fixed rodents during in vivo patch clamp recordings. Our method uses an algorithm based on optical flow and point-process filtering which can be run on images acquired on regular cameras at low frame-rates. We show that the algorithm can achieve at least comparable accuracy in detecting LOC when compared with consensus among human observers, as well as improved precision when compared with individual observers. In the future, we aim to expand the method to detect more behavioral end-points during anesthesia such as paradoxical excitation. Eventually, we hope to enable multi-modal anesthesia studies, which incorporates behavioral and neurophysiological data.

Index Terms—anesthesia, optical flow, point-process, motion detection, image feature extraction.

I. INTRODUCTION

Precisely identifying loss of consciousness (LOC) is key to studying its neural correlates. In clinical practice, simple tests such as responsiveness to verbal commands are often used. In research settings, more complex tests such as response to combinations of auditory stimuli can be used to define LOC [1]. These behavioral assays are used in multi-modal anesthesia studies in combination with with EEG, fMRI, and other physiological recordings [2] to examine mechanisms by which anesthetic drugs produce LOC.

In contrast to humans, the repertoire of behavioral assays for LOC in rodents is much more limited. The standard measure is the Loss of Righting Reflex (LORR), which is assessed by quickly placing the animal in the supine position every minute until it fails to right itself [3]. While LORR is a reliable measure, it is incompatible with techniques with high temporal and spatial resolutions that can be used in rodents to obtain even more insights about anesthetic actions and LOC. For example, targeted multisite recordings can be used to assess the temporal dynamics of large cortical and subcortical networks during LOC [4]; in vivo patch clamp recordings [5] can be used to study the effects of anesthetics on membrane potential, and invasive neuronal imaging techniques such as calcium imaging [6] can be used to produce highly detailed observations of neurophysiological dynamics during anesthesia. These methods all require that the animal be head-fixed, which precludes the possibility of performing the LORR assessment.

We propose a new behavioral assay to assess LOC in head-fixed rodents using the loss of movement (LOM) in whiskers and paws. This proposal is inspired by previous work in which we established a strong correlation between LORR and LOM [4]. We demonstrate a proof-of-concept of this new assay in head-fixed rodents undergoing in vivo whole-cell current clamp recordings. Because LOM in whiskers and paws is challenging to detect by visual inspection, we developed an algorithm based on optical flow and point-process filtering to perform this task automatically.

II. METHODS

A. Animal preparation and video acquisition

All procedures were performed in agreement with federal, state, and local regulations. The protocol #0113-008-16 was approved by the Committee on Animal Care at the Massachusetts Institute of Technology. The methods have been published in detail in Kodandaramaiah et al. [5]. In short, adult male C57BL/6 mice, 8 weeks old, were implanted with metallic head plates under anesthesia and sterile conditions. After a week of post-surgical recovery, where analgesics were administered, the mice were behaviorally acclimated to the head-fixed set-up in sessions of increasing duration from 10 minutes to 30 minutes over a period of 7 days. Mice were given diluted condensed milk during the acclimation sessions...
to minimize stress and provide positive reinforcement. On the
day of the experiment, an indwelling cannula was placed in
the peritoneal cavity, and the mice were placed in the head-
fixed recording set-up for in vivo whole-cell current clamp
[5].

A scientific grade camera (Firefly MV 0.3 MP Mono
FireWire 1394a, Flir Machine Vision) was installed in front
of the mice, together with an off-the-shelf infrared illumina-
tion source. The camera’s shutter was triggered at 20
frames per second using a computer interface board (NI-USB
6259, National Instruments) controlled via custom-
written LabView Software (National Instruments). Frames
were acquired at a resolution of 800×600 pixels. A copy
of the trigger signal was sent to the patch amplifiers for
synchronization with the neurophysiological recordings. The
full set-up for the experiment and video acquisition is shown
in Fig. 1.

The video acquisition was started simultaneously with the
cellular recordings. Four minutes of baseline data were ac-
quired before the mice were dosed with either dexmedetomi-
dine (200 mcg/kg) or ketamine (150 mg/kg). The recordings
continued for a variable period until the whole-cell state was
lost.

B. Movement detection using optical flow analysis

We used optical flow to extract movement of whiskers and
paws from the videos acquired. To do so, we first manually
marked the regions of interest (ROIs) in the videos to include
either the right paw or the right whiskers. The head-fixed set-
up constrained the ROIs to a fixed area while allowing full
range of movement for the whiskers and front paws. Next, we
sub-sampled the ROI by a factor of two, and computed the
optical flow using the Lucas-Kanade Difference-of-Gaussian
algorithm as implemented in MATLAB [7], [8]. We set the
value of 3 because the original video was acquired at a low
number of frames for temporal smoothing to the minimum
algorithm as implemented in MATLAB [7], [8]. We set the
optical flow using the Lucas-Kanade Difference-of-Gaussian
algorithm. D) Horn-Schunk algorithm. The scaling factor for the
vectors produced by the conventional Lucas-Kanade algorithm is a fourth
of the scaling factor applied to the vectors produced by the other two
algorithms.

Fig. 2. Typical examples of optical flow vectors (green arrows) computed
applying three different algorithms. A) ROI corresponding to the front paws. B) Lucas-Kanade Difference-of-Gaussian algorithm. C) Conventional Lucas-
Kanade algorithm. D) Horn-Schunk algorithm. The scaling factor for the
vectors produced by the conventional Lucas-Kanade algorithm is a fourth
of the scaling factor applied to the vectors produced by the other two
algorithms.

magnitude of the optical flow vectors within each ROI, \( y_{i,\tau} \)
(where \( i = w \) for whiskers and \( i = p \) for paws), to summarize
the optical flow measurements.

Next, we adapted a previously validated recursive variance
algorithm (RVS) to convert \( y_{i,\tau} \) into binary signals where
zeros represent non-movement and ones represent movement
[9]. This algorithm computes a recursive estimate of the local
signal variance and threshold the estimate as follows:

\[
\begin{align*}
\mu_{i,\tau} &= \beta \mu_{i,\tau-1} + (1 - \beta) y_{i,\tau} & (1a) \\
v_{i,\tau}^2 &= \beta v_{i,\tau-1}^2 + (1 - \beta) (y_{i,\tau} - \mu_{i,\tau})^2 & (1b) \\
b_{i,\tau} &= \delta[v_{i,\tau}^2 < \theta] & (1c)
\end{align*}
\]

here \( y_{i,\tau} \) is the median magnitude of the optical flow vectors,
\( \mu_{i,\tau} \) is its local mean, \( v_{i,\tau}^2 \) is its local variance, and \( b_{i,\tau} \) is the
binary signal for whiskers \( (i = w) \) or paws \( (i = p) \) at time \( \tau \).
The algorithm has two parameters: the “forgetting factor” \( \beta \)
which determines the relative influence of current data and
past estimate on the current estimates; and the classification
threshold \( \theta \) which determines the outcome of the indicator
function \( \delta[\cdot] \) (which is 1 if the inequality is satisfied and 0
otherwise). We set the value of \( \beta \) to the globally-optimal
value reported in [9] and customized the value of \( \theta \) for
each data set. Specifically, we applied a bisecton search
algorithm to find the threshold \( \theta \) such that at baseline
the total duration of movement detected was between 25–75%.

Fig. 3. State-space model for estimating the movement probabilities given
median optical flow data.
C. Computing the movement probability (MVP)

We introduce the concept of movement probability (MVP) of the whiskers and paws which describes the instantaneous probability for movement. This is a statistically rigorous way to generate a denoised and scale-free measure from binary data [10] and can be used to classify the behavior states into LOC or non-LOC. MVP is derived based on a state-space model as shown in Fig. 3. Let $\Delta_p$ be the sampling period of MVPs and $\Delta_b$ be the sampling period of the binary observations. In our model, the MVP (denoted as $p_t$) drives the generation of the binary observations through a binomial probability model:

$$f(p_t) = \binom{N}{k_t} p_t^{k_t} (1 - p_t)^{N-k_t},$$

where $N = \Delta_p/\Delta_b$ and $k_t = \sum_{j=t-(t-1)N}^{tN} b_j$, $p_t$ also relates to an underlying state $z_t$ through a sigmoid function

$$g(z_t) = p_t = \frac{1 - \exp(-z_t)}{1 + \exp(-z_t)}.$$

We model the dynamics of the states as a random walk process:

$$h(z_{t-1}) = z_t = z_{t-1} + \epsilon_t$$

where $\epsilon_t$ are independent Gaussian random variables with mean 0 and variance $\sigma^2$. This definition of the state transition provides a stochastic continuity constraint to ensure that the states (and hence the MVPs) that are close in time are close in value. We adapted the algorithm for estimating the MVPs from our previous work on point-process filters [11]. The key steps of the filtering algorithm are shown below:

- **Prediction:**
  $$z_{t|t-1} = z_{t-1|t-1} + \sigma^2_{t|t-1}$$
  $$\sigma^2_{t|t-1} = \sigma^2_{t-1|t-1} + \sigma^2$$

- **Update:**
  $$z_t = z_{t|t-1} + \sigma^2_{t|t-1} \frac{dp_t}{dz_t} \bigg|_{z_{t|t-1}}$$
  $$\sigma^2_{t|t} = \sigma^2_{t|t-1} + g_t^2 [p_{t|t-1}(1 - p_{t|t-1})]^{-1}$$

where

$$z_{t|t-1} = \mathbb{E}[z_t|z_{t-1}, \sigma^2, z_0]$$

$$\sigma^2_{t|t-1} = \mathbb{E}[(z_t - z_{t|t-1})^2|z_{t-1}, \sigma^2, z_0]$$

$$z_t = \mathbb{E}[z_t|k_t, \sigma^2, z_0]$$

$$\sigma^2_t = \mathbb{E}[(z_t - z_t)^2|k_t, \sigma^2, z_0]$$

$$\ell_t = k_t - np_{t|t-1}$$

$$dp_t = \frac{x_t(1 - p_t)}{\exp(x_t) + 1}$$

$$dz_t = \frac{x_{t|t-1}(1 - p_{t|t-1})}{\exp(x_{t|t-1}) + 1}$$

$$g_t = \frac{z_{t|t-1} \exp(x_{t|t-1})}{\exp(x_{t|t-1}) + 1}(1 - p_{t|t})$$

We computed MVPs separately for whiskers and paws. To initialize the algorithm, an EM-algorithm was used on the four minutes of baseline data to find parameters $\sigma^2$ and $z_0$.

D. Identification of LOC and performance assessment

We defined LOC as having a MVP of less than 0.1 in either whiskers or paws and identified the period of LOC in each animal using the fully automated algorithm described in Sections II-B and II-C. We also defined the minimal time for a period of LOC identified to be at least 3 minutes. We compared the performance of the algorithm to the annotations of LOC and non-LOC periods provided by three independent and experienced human observers who were provided with the videos only. We defined human consensus as having agreement between at least two of the three observers. Then we computed the percentage time when there was inter-human $A_{hh}$, human-algorithm $A_{ha}$, and algorithm-human consensus agreement $A_{ac}$ on the labels of LOC or non-LOC. From this analysis, we obtain 7 comparisons. Each of these is characterized by a distribution of the percentage time in which the pair compared was in agreement. Finally, we computed the 95% confidence interval for the median value of each of these distributions using non-parametric bootstrap.

We defined two distributions as statistically significant only when there is no overlap between the confidence interval of their median values.

III. RESULTS

We conducted 10 experiments in total. Dexmedetomidine was used in one half of the experiments and ketamine in the other half. The duration of the experiments ranged from 19.7–48.5 min with a median of 30.2 min. We processed the videos using the algorithms described in Sections II-B and II-C, and automatically identified periods of LOC using the criteria described in Section II-D. Figure 4 shows the whisker and paw median optical flow, movement detected, and MVP, as well as the LOC identified by the algorithm and human observers for one experiment.
We show a box plot summarizing inter-human agreement $A_{hh}$, human-algorithm agreement $A_{ha}$, and algorithm-human consensus agreement $A_{ac}$ for all experiments in Fig 5. The whisker length is set to 1.5 times of the interquartile range (IQR). Table 1 summarizes the median, 95% confidence interval (CI) of the median, mean, and standard deviation (std) for each of the seven comparisons. None of the comparisons made were statistically different from the others. However, we can see that $A_{ac}$ has the smallest 95% CI of the median and standard deviation while $A_{23}$ has the largest 95% CI of the median and standard deviation. Interestingly, the agreement between the algorithm and human consensus was less variable than the agreement between the individual human observers and human consensus. Therefore, the results indicate that our algorithm perform at least as well as human observers, and has improved precision when compared with individual human observers.

IV. DISCUSSION AND CONCLUSION

We propose a new behavioral assay based on LOM for examining LOC in head-fixed rodents where the traditional assays such as LORR cannot be used. As evident from the variability of the inter-human agreement in our results, the task of identifying LOM in whiskers and paws is challenging. This is probably because such movements are much less obvious and harder to assess than LORR. The process of having humans manually determine LOC by watching the video is also labor intensive and subjected to bias [12]. Therefore we are motivated to develop an automated algorithm to perform this task.

We designed and implemented an algorithm based on optical flow metrics of motion and point-process filtering to estimate a novel index called movement probability. We used the movement probability to detect LOC in 10 experiments, and confirmed that the proposed behavior assay based on LOM is tractable. By comparing with human annotated period of LOC versus non-LOC, we showed that the algorithm is as accurate and more precise. The algorithm was also applied successfully to recordings made at low frame-rates and moderate resolution. Therefore, it is useful without using cameras with high resolution and frame-rates.

In this analysis, we have limited ourselves to identifying periods of LOC vs non-LOC for an initial proof-of-concept. However, the movement probability is not a binary measure but a continuous metrics and can be used to characterized more subtle behavioral changes. This can be exploited in future work to refine our current analysis of LOC, distinguish between different depths of sedation during anesthesia, and characterize other relevant behavioral states such as paradoxical excitation.

Finally, our proposed method will enable the combination of behavioral and invasive neurophysiological measures in head-fixed rodents. This is crucial for gaining new insights about the neural mechanism of anesthesia and consciousness.

REFERENCES


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