Algorithm for Automatic Behavior Quantification of Laboratory Mice Using High-Frame-Rate Videos

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Abstract: In this paper, we propose an algorithm for automatic behavior quantification in laboratory mice to quantify several model behaviors. The algorithm can detect repetitive motions of the fore- or hind-limbs at several or dozens of hertz, which are too rapid for the naked eye, from high-frame-rate video images. Multiple repetitive motions can always be identified from periodic frame-differential image features in four segmented regions—the head, left side, right side, and tail. Even when a mouse changes its posture and orientation relative to the camera, these features can still be extracted from the shift- and orientation-invariant shape of the mouse silhouette by using the polar coordinate system and adjusting the angle coordinate according to the head and tail positions. The effectiveness of the algorithm is evaluated by analyzing long-term 240-fps videos of four laboratory mice for six typical model behaviors: moving, rearing, immobility, head grooming, left-side scratching, and right-side scratching. The time durations for the model behaviors determined by the algorithm have detection/correction ratios greater than 80% for all the model behaviors. This shows good quantification results for actual animal testing.

Key Words: automatic behavior quantification, high-speed vision, polar transform, new drugs development.

1. Introduction

Laboratory animals such as mice and rats play important roles in applied research for new drug development and in disease psychopharmacology, toxicology testing, and other biomedical research. In animal testing, in vivo behavior analysis of laboratory animals is necessary to quantify various types of model behaviors for disorders that indicate the degrees of disease. Among these model behaviors, there are many types of high-speed model behaviors such as scratching and grooming, which involve repetitive motions of the fore- or hind-limbs at several or dozens of hertz; these motions are much faster than human leg motion. Thus, several high-speed model behaviors involving repetitive motions of the limbs are difficult to observe and objectively quantify with the naked eye or even a standard-frame-rate (30-fps) NTSC video camera.

The periodic leg motions of humans have also been studied by many researches, and a considerable amount of research on automatic gait characteristics analysis based on image analysis has been conducted [1]–[3]. Most of these studies consider periodic human gait patterns with frequencies on the order of several hertz and analyze video images captured at a standard video rate of 30 fps or less. However, scratching behavior in mice is a typical high-speed model behavior specially induced by itching, wherein they scratch their head or other parts of their body with their hindlimbs at dozens of hertz. Thus, a high-frame-rate (HFR) video analysis becomes effective for accurate and objective quantification of high-speed model behaviors of mice involving rapid motions of their limbs, because these motions are too rapid for the naked eye and the NTSC video cameras. The authors have already proposed a behavior quantification algorithm [4] for scratching based on HFR video and have developed a real-time 240-fps image analysis system [5] dedicated to long-term quantification of scratching behaviors of laboratory animals. In this approach, it is assumed that scratching is a single behavior to be quantified, although there are several cases in animal testing where other model behaviors occur simultaneously, involving similar repetitive behaviors such as grooming with the forelimbs, which is often misinterpreted as scratching.

Hence, in this paper, we propose an HFR-video-based behavior quantification algorithm to detect many types of model behaviors of laboratory mice, including repetitive behaviors, at several or dozens of hertz. The algorithm can segment repetitive limb movements into four regions—head, left side, right side, and tail—by analyzing a mouse silhouette in a polar coordinate system. Thus, multiple model behaviors can always be quantified independently of the position and orientation. In this study, the proposed algorithm is applied to 240-fps videos of several ICR mice to evaluate its effectiveness. The time durations of six typical behaviors are calculated and the effectiveness is determined on the basis of the detection/correction ratio. The six typical model behaviors include left-side and right-side scratching and head grooming.

2. Related Works

In animal tests for behavior pharmacology, laboratory mice exhibit many types of behaviors with specific motion features, including quick movements of their limbs. However, the behaviors are often observed by the human naked eye and manually quantified. The result is often inaccurate and nonobjective notwithstanding the arduous labor required, which makes the process inefficient. This is because movements of the fore- and hindlimbs are sometimes too fast for the naked eye, and the
quantified results are strongly subject to the observer’s experience.

Several automatic behavior quantification algorithms and systems have been already developed for objective behavior analysis and quantification of repetitive movements of limbs such as scratching at dozens of hertz. Elliott et al. [6] relied on invasive observation using a magnet sensor embedded under the skin of the animal and quantified scratching behavior by applying frequency analysis. However, the embedded marker may induce stress. Umeda et al. [7] reported an acoustic sensor system for counting repetitive limb movement, which does not require planting any marker, but requires a special setting to eliminate extraneous sound. LABORAS [8] is a commercial sensor platform for laboratory animals that is based on force transducers. It can extract vibrating behaviors of animals without the need for attachment of any sensor on the animals. Systems based on such nonvisual sensor technology are advantageous in that they are noninvasive and independent of lighting conditions. However, their limitations in space resolution make it difficult to identify the shapes of the animals for precise behavior detection, and there are not always suitable for discrimination of similar repetitive behaviors such as scratching and grooming. The same problem exists in other systems based on nonvisual sensors.

By contrast, video images can provide higher-spatial-resolution information than nonvisual sensor technology. For behavior analysis of laboratory animals, several methods have been proposed to extract body-parts-based features for behavior recognition. Liang et al. [9] proposed a labeling method for animal body parts and extracted their movements for multiple behavior detection. Crook et al. [10] identified semi-invariant features in mouse contours for robust behavior recognition. Fröhlich et al. [11] applied advanced machine learning methods to analyze rat behavior. However, all these vision-based methods relied on standard video images at 30 fps or less, which is not always sufficient for precise behavior analysis of rapid and repetitive movements of the limbs. To solve this problem, Ishii et al. [4],[5] performed precise scratching behavior quantification by introducing HFR video analysis and extracting repetitive movements of the limbs at several or dozens of hertz. In this approach, frame-to-frame difference features for the entire image were calculated for detecting the repetitive movements involved in scratching. However, this system still cannot discriminate multiple behaviors of laboratory mice, because it does not consider the specific body parts that are related to the behavior.

Grooming behavior refers to the actions of licking the fore- and hindlimbs, back, belly, or groin. It is a well-known model behavior that indicates stress and anxiety [12],[13], and it is relevantly different from scratching. Moreover, a mouse generally scratches by using its dominant hindlimb as its disorder-evoked behavior, and it had been reported [14],[15] that the degree of relaxation can be determined by discriminating and quantifying left-side right-side scratching behavior. Thus, quantification of model behaviors such as grooming, left-side scratching, and right-side scratching is necessary for more precise behavior quantification of laboratory mice. This can be achieved by detecting when and where a mouse conducts performs repetitive limb movements using HFR video analysis. For behavior classification, a method which is categorized into pattern recognition based on interpretation trees is proposed [16]–[18].

3. Proposed Algorithm

In this paper, we propose an improved algorithm for multiple behavior quantification in laboratory mice by means of HFR video analysis. This algorithm detects when and where a mouse performs repetitive movements of its limbs at dozens of hertz in HFR videos and quantifies these behaviors by calculating the frame-to-frame difference features in four segmented regions—the head, the left side, the right side, and the tail. The algorithm can quantify multiple model behaviors independently of the position and orientation of the animal by analyzing its silhouette in a polar coordinate system, whose angular coordinate is adjusted according to the head and tail positions. Our algorithm is divided into three parts, as shown in Fig. 1: A) Polar transform for silhouette contour extraction B) Shift- and orientation-invariant frame-to-frame difference feature calculation C) Behavior discrimination using a behavior look-up table.

In this study, six model behaviors were detected using frame-to-frame difference features in the four segmented regions: moving, rearing, immobility, head grooming, left-side scratching, and right-side scratching. Here moving refers to the state of walking or running; rearing involves sitting on the hindlimbs; immobility refers to staying still for a brief period of time. Head grooming refers to the brisk movement of the forelimbs around the head. Left- or right-side scratching is the repetitive movement of the left or right hindlimb when scratch the head or other body parts. These behaviors are discriminated by using a behavior look-up table (described below), defined by shift- and orientation-invariant frame-to-frame difference features. The relevant flowchart for the behavior quantification algorithm in this study is illustrated in Fig. 2.

3.1 Polar Transform for Silhouette Contour Extraction

The shape information of a mouse is extracted from the HFR video images in the form of silhouette contours, expressed in the polar coordinate system $(r, \theta)$. We assume that the HFR videos are captured from the top view.
where \( \tilde{\theta} \) is the angular coordinate at time \( t \) for all the pixels \( (r, \theta, t) \), the inner boundary is extracted as a silhouette contour in the polar coordinate system.

\[
\delta(x) = \begin{cases} 
1 & \text{(if } x = 0) \\
0 & \text{(otherwise)} 
\end{cases}
\]

(5) Contour extraction

By calculating the minimum distance \( r_m(\theta, t) \) for all the pixels on \( P(r, \theta, t) \) in the direction of \( \theta \), the inner boundary is extracted as a silhouette contour in the polar coordinate system.

\[
r_m(\theta, t) = \arg \min_P P(r, \theta, t).
\]

### 3.2 Frame-to-Frame Difference Features Calculation

Frame-to-frame difference features for the segmented regions are used as shift- and orientation-invariant features for robust behavior recognition against frequent posture changes. The tail and head are detected as standard points for the redefined polar coordinate system.

(1) Tail detection

Around the tail, the silhouette contour \( r_m(\theta, t) \) is greatly curved and has a sharp distribution in a specific direction. Thus, the tail direction \( \theta_t(t) \) can be determined as follows:

\[
\theta_t(t) = \arg \max_{\theta} \left| \frac{\partial r_m(\theta, t)}{\partial \theta} \right| \left( \frac{\partial r_m(\theta, t)}{\partial \theta} > T_i \right),
\]

where \( h(\theta, t) \) is the number of pixels of \( P(r, \theta, t) = 1 \) in the direction \( \theta \sim \theta + d\theta \).

(2) Head detection

Next, the head direction \( \theta_h(t) \) can be determined as the most distant point from the tail in the silhouette contour \( r_m(t) \):

\[
\theta_h(t) = \arg \max_{\theta \in \Theta_t} r_m(\theta, t),
\]

where \( \Theta_t = \{ \theta : \theta - \Theta_h/2 \leq \theta < \theta + \Theta_h/2 \} \) is assumed as being the tail region of range \( \Theta_h \) in the tail direction \( \theta_t \).

(3) Body-parts segmentation

To negate the effects of bending by the mouse and its variable orientation, the angular coordinate \( \phi \) is redefined from \( \theta \) using the detected tail and head directions as follows:

\[
\phi = \frac{\pi}{\Phi^+} (\min(\theta_t, \theta_h) - \theta) \quad (0 \leq \theta < \min(\theta_t, \theta_h))
\]

\[
\phi = \frac{\pi}{\Phi^-} (\theta - \min(\Theta_t, \theta_h)) \quad (\min(\theta_t, \theta_h) \leq \theta < \max(\Theta_t, \theta_h)),
\]

\[
\phi = 2\pi - \Phi^- \quad (\max(\Theta_t, \theta_h) \leq \theta < 2\pi)
\]

where \( \Phi^+ = |\theta_h(t) - \theta_t(t)| \) and \( \Phi^- = 2\pi - \Phi^+ \).

Then, the silhouette contour \( r_m(\theta, t) \) can be converted into the shift- and orientation-invariant contour \( r_m(\phi, t) \) in the redefined polar coordinate system. This contour is segmented into four regions, the head \( R_h \), the left side \( R_l \), the right side \( R_r \), and the tail \( R_t \), as follows:

\[
R_h = \{ \phi : \phi - \Phi_h/2 \leq \phi < \Phi_h/2 \},
\]

\[
R_l = \{ \phi : \phi + \Phi_l/2 \leq \phi < \Phi_l/2 \},
\]

\[
R_r = \{ \phi : \Phi_r/2 \leq \phi < \phi + \Phi_r/2 \},
\]

\[
R_t = \{ \phi : \phi + \Phi_h/2 \leq \phi < 2\pi - \Phi_h/2 \},
\]
where the center directions and ranges of the tail region \( R_t \) are set to 0 and \( \Phi_t \), and those of the head region \( R_h \) are set to \( \pi \) and \( \Phi_h \), respectively.

(4) Frame-to-frame differencing for segmented regions
The frame-to-frame difference features \( F_i(t), F_r(t), F_h(t) \), and \( F_b(t) \) are each calculated for the segmented regions \( R_t, R_r, R_h, \) and \( R_b \), respectively, as motion-based features:

\[
F_i(t) = \int_{\phi \in \Phi_i} [f_\phi(t + dt) - f_\phi(t)] d\phi \quad (i = t, r, h, l),
\]
where \( dt \) is the time interval for frame differencing.

### 3.3 Behavior Discrimination with a Look-up Table

Finally, the six model behaviors involving quick limb movements are quantified by sequentially executing the subprocesses for extracting the shift- and orientation-invariant frame-to-frame difference features in the following order: moving, rearing, immobility, head grooming, left-side scratching, and right-side scratching.

(1) Moving detection
Moving is detected by thresholding the speed of the entire body, \( V(t) \), with a threshold \( T_{mv} \) as follows:

\[
q_{mv}(t) = \begin{cases} 1 & (\text{if } V(t) > T_{mv}) \\ 0 & (\text{otherwise}) \end{cases},
\]
where the speed \( V(t) \) is defined as follows:

\[
V(t) = \sqrt{\left(\frac{\partial c_x}{\partial t}\right)^2 + \left(\frac{\partial c_y}{\partial t}\right)^2},
\]

(2) Rearing detection
Rearing is detected by using the area of the entire body, \( A(t) \), with a threshold \( T_{re} \) as follows:

\[
q_{re}(t) = \begin{cases} 1 & (\text{if } A(t) < T_{re}) \\ 0 & (\text{otherwise}) \end{cases},
\]
where the area \( A(t) \) is calculated as follows:

\[
A(t) = \int_{\phi} f_\phi(t) d\phi.
\]

(3) Immobility detection
Immobility is detected by using the frame-to-frame difference feature for the entire body, \( F(t) \), with a threshold \( T_{im} \),

\[
q_{im}(t) = \begin{cases} 1 & (\text{if } F(t) < T_{im}) \\ 0 & (\text{otherwise}) \end{cases},
\]
where \( F(t) \) is calculated as follows:

\[
F(t) = F_i(t) + F_r(t) + F_h(t) + F_b(t).
\]

(4) Head grooming detection
In order to detect head grooming, the repetitive pulse detection algorithm described in [5] is adopted for the frame-to-frame difference feature \( F_h(t) \) for the head region:

i) Pulse thresholding
\( p_{hg}(t) \) are calculated as pulses by thresholding \( F_h(t) \) to determine the presence or absence of motion as follows:

\[
p_{hg}(t) = \begin{cases} 1 & (\text{if } F_h(t) > T_{p0}) \\ 0 & (\text{otherwise}) \end{cases},
\]
where \( T_{p0} \) is the threshold for removing small movements.

ii) Short-pulse detection
\( s_{hg}(t) \) are extracted as repetitive short pulses from \( p_{hg}(t) \), while other large or long-term pulses are excluded.

\[
s_{hg}(t) = \begin{cases} 1 & (d(t) < \tau_0) \\ 0 & (\text{otherwise}) \end{cases},
\]
where \( d(t) \) is the duration for \( s_{hg}(t) = 1 \) involving time \( t \), and \( \tau_0 \) is the threshold for long-term pulse rejection.

iii) Long-pulse detection
The short intervals in \( s_{hg}(t) \) are compensated for by combining the repetitive pulses into a single long pulse \( s'_{hg}(t) \) as follows:

\[
s'_{hg}(t) = \begin{cases} 1 & (d' < \tau_1) \\ 0 & (\text{otherwise}) \end{cases},
\]
where \( d' \) is the time duration for \( s_{hg}(t) = 0 \), and \( \tau_1 \) is the threshold to determine whether the pulses are compensated.

Head grooming is analyzed when the duration \( d''(t) \) of \( s'_{hg}(t) = 1 \) is greater than \( \tau_2 \) as follows:

\[
q_{hg}(t) = \begin{cases} 1 & (d'' > \tau_2) \\ 0 & (\text{otherwise}) \end{cases},
\]

(5) Left-side scratching detection
The state \( q_{sl}(t) \) of left-side scratching is determined from the frame-to-frame difference feature \( F_l(t) \) for the left side in the same manner as that for head grooming detection.

(6) Right-side scratching detection
The state \( q_{sr}(t) \) of right-side scratching is determined with the frame-to-frame difference feature \( F_r(t) \) for the right side in the same manner as that for head grooming detection.

(7) Quantification of discriminated behaviors
The durations \( Q_{sl}(t; t_1, t_2) \) between \( t = t_1 \) and \( t = t_2 \) for all the discriminated behaviors are calculated by integrating the time for \( q(t) = 1 \) in the following:

\[
Q_{sl}(t_1, t_2) = \int_{t_1}^{t_2} q(t) dt = \int_{t_1}^{t_2} \left( q_{mv}(t) + q_{re}(t) + q_{im}(t) + q_{hg}(t) + q_{sl}(t) + q_{sr}(t) \right) dt.
\]

The look-up table with image features for behavior discrimination in this study can be summarized as in Table 1. The vertical axis describes the image features to be thresholded, and the behaviors are sequentially discriminated from left to right in the table. Here, “O” and “X” represent large and small values, respectively, and “-” indicate values not related with the behavior.

### 4. Experiments for Laboratory Mice

#### 4.1 Experimental Settings

The proposed algorithm was tested in several experiments on long-term HFR videos of laboratory mice. Figure 3 shows the

<table>
<thead>
<tr>
<th>Behavior</th>
<th>MV</th>
<th>RE</th>
<th>IM</th>
<th>HG</th>
<th>SL</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>body speed ( V(t) )</td>
<td>O</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>body area ( A(t) )</td>
<td>-</td>
<td>-</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>FD for entire body ( F(t) )</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>FD for head ( F_h(t) )</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>O</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>FD for left-side ( F_l(t) )</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>FD for right-side ( F_r(t) )</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>O</td>
</tr>
</tbody>
</table>

configuration of the experimental system used for recording the HFR videos. In the system, an HFR camera was installed 80 cm above the floor of the cage. The cage was set on a flat infrared (IR) illuminator with the dimensions 30 cm × 30 cm for clearly capturing the silhouette of the mouse. These dimensions are larger than those of the bottom of the cage, and the peak wavelength of the IR illuminator is 950 nm. A transparent acrylic cage was used that were 12 cm × 20 cm × 40 cm in size.

The HFR camera used was a INCS1010, as shown in Fig. 4, which is designed for long-term HFR video recording. It can capture and output 320 × 400 pixel images at 240 fps, and transmits two consecutive frames together as one 640 × 400 pixel image. The combined images are output via 4-channel NTSC analog outputs at a frame rate of 30 fps. Here, the frame timing shifts between the NTSC analog outputs were set to 1/120 s. The video was acquired by four PC-MV7DX/U2 MPEG encoders (Buffalo Inc.) and stored on a TeraStation network-attached storage system (Buffalo Inc.). The stored MPEG files were then accessed on a personal computer (PC) via LAN, and the long-term HFR video sequences were then generated by combining the four stored MPEG-2 files on the personal computer. For 4-channel images synchronization, the INCS1010 outputs images were provided a 15-bit binary time code on the upper right corner. The code indicated the original frame timing of the 320 × 400 pixel frames captured at 240 fps.

In the experiments, the field of view of the INCS1010 camera was set as 350 mm × 438 mm, which covers the entire region in which the mouse can move; a pixel corresponds to 0.91 mm. The distance from the camera to the mouse was 75 cm. The recorded HFR video sequences were analyzed on the PC. Here the behavior quantification algorithm was implemented as offline after capturing the HFR videos.

With this setup, we recorded HFR videos of four 5-week-old male ICR mice, namely, ICR1, ICR2, ICR3, and ICR4. The ICR mouse is a Swiss mouse widely used in oncological and pharmaceutical research. The weight of each mouse was approximately 27 g, and the body length including its tail was approximately 8 cm. The mice were put into the cages without any prior treatment. For each mouse, the 320 × 400-pixel video was recorded at 240-fps for 20 min. In the experiments, the parameters for behavior quantification were set to the following values:

- **Polar transform for silhouette contour extraction**, scale for opening operation, \( r_0 = 9 \).
- **Frame-to-frame difference features calculation**
  - excluded range in head detection: \( \Theta_a = \pi/3 \),
  - range of tail region: \( \Phi_t = \pi/3 \),
  - range of head region: \( \Phi_h = \pi/3 \),
  - interval of frame differencing: \( dt = 42 \) ms.
- **Behavior discrimination with a behavior look-up table**
  - moving detection: \( T_{mv} = 100 \) pixel/s,
  - rearing detection: \( T_{re} = 7.4 \times 10^5 \) pixel,
  - immobility detection: \( T_{im} = 5.3 \times 10^3 \) pixel,
  - head grooming detection: \( T_{pt} = 100 \) pixels, \( \tau_0 = 83 \) ms, \( \tau_1 = 167 \) ms, \( \tau_2 = 208 \) ms,
  - left-side / right-side scratching detection: \( T_{pt} = 250 \) pixels, \( \tau_0 = 21 \) ms, \( \tau_1 = 83 \) ms, \( \tau_2 = 208 \) ms.

These parameters were carefully adjusted, after some suggestions from experienced observers. All the parameters were applied to the four experiments on the ICR mice.

### 4.2 Quantification Results

Figure 5 shows the 20-min analysis results for ICR1. In this figure, automatically discriminated results for the six model behaviors—moving (MV), rearing (RE), immobility (IM), head grooming (HG), left-side scratching (SL), and right-side scratching (SR)—were plotted with six image features for behavior discrimination, \( V(t) \), \( A(t) \), \( F(t) \), \( F_h(t) \), \( F_l(t) \), and \( F_r(t) \). Here OT refers to undiscriminated behavior.

For moving, Fig. 6 (a) shows magnified graphs of image features, \( V(t) \), \( A(t) \), and \( F(t) \) for \( t = 4^35^5^5^0^0^0^0 \) to \( 4^36^5^5^0^0^0^0^0 \) in 1 s. Figs. 6 (b)–(d) show a sequence of input images \( I(x, y, t) \), silhouette contours \( r_m(\phi, t) \), and frame-to-frame difference features \( f(\phi, t) = |r_m(\phi, t) - r_m(\phi, t - dt)| \), taken at intervals of 0.033 s. For rearing, Fig. 7 (a) shows graphs of \( V(t) \), \( A(t) \), and \( F(t) \) for \( t = 5^5^6^6^0^0^0^0 \) to \( 5^56^6^5^0^0^0^0 \) and (b)–(d) show a sequence of \( I(x, y, t) \), \( r_m(\phi, t) \), and \( f(\phi, t) \). For immobility, Fig. 8 (a) shows graphs of \( V(t) \), \( A(t) \), and \( F(t) \) for \( t = 14^38^3^0^0^0^0 \) to \( 14^39^8^0^0^0^0 \), and (b)–(d) show a sequence of \( I(x, y, t) \), \( r_m(\phi, t) \), and \( f(\phi, t) \). As shown in Figs. 6–8, the large body motion was interpreted as moving by thresholding the entire body’s speed \( V(t) \) with a threshold \( T_{mv} \) of 100 pixel/s. The apparent body shrinking was interpreted as rearing by thresholding the entire body’s area \( A(t) \) with a threshold \( T_{re} \) of 7.4 × 10^5 pixel. And no movement was interpreted by the frame-to-frame difference feature \( F(t) \) as immobility by thresholding the frame-to-frame difference feature of the entire body \( F(t) \) with a threshold \( T_{im} \) of 5.3 × 10^3 pixel.

For head grooming, Fig. 9 (a) shows magnified graphs of frame-to-frame difference features for segmented regions,
Figures 9 (b)–(d) show a sequence of input images $I(x, y, t)$, silhouette contours $\tilde{r}_m(\phi, t)$, and frame-to-frame difference features $f(\phi, t)$. As shown in Fig. 9, silhouette contours obtained were invariant regardless of the orientation and posture bending of the mouse, and the frame-to-frame difference features of the head region $F_{h}(t)$ were localized for detecting the limb movement around the head region. Figure 9 indicates that head grooming was identified 8 times within 1 s from the repetitive pulses $F_{h}(t)$. These repetitive motions around the head region were discriminated as head grooming.

Figure 10 (a) shows graphs of $F_{h}(t)$, $F_{l}(t)$, and $F_{r}(t)$ for $t = 7'51''00$ to $7'52''00$ in the case of left-side scratching, and (b)–(d) show a sequence of $I(x, y, t)$, $\tilde{r}_m(\phi, t)$, and $f(\phi, t)$. Figure 10 indicates that left-side scratching was observed 13 times in 1 s from the repetitive pulses of the frame-to-frame differ-
ence features on the left-side region, $F_l(t)$. Thus, these repetitive motions around the left-side region were interpreted as left-side scratching in a manner similar to that for head grooming detection.

In the 20-min experiment for ICR1, right-side scratching was not frequently observed because the mouse had a strong tendency to scratch with its dominant left hindlimb. The similar tendency, scratching with the dominant limb, was also observed in the other experiments, where the dominant limbs for ICR2, ICR3, and ICR4 were the right, right, and left, respectively. For right-side scratching, Fig. 11 (a) shows graphs of $F_h(t)$, $F_l(t)$, and $F_r(t)$ for $t = 0'56''00$ to $0'57''00$ in the experiment on ICR2, and (b)–(d) show a sequence of $I(x,y,t)$, $\tilde{\tau}_m(\phi,t)$, and $F_r(t)$.
Fig. 12 Discriminated time durations (ICR1 ∼ ICR4).

**Table 2** Time durations of discriminated behaviors (all).

<table>
<thead>
<tr>
<th>auto</th>
<th>MV</th>
<th>RE</th>
<th>IM</th>
<th>HG</th>
<th>SL</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>783</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>RE</td>
<td>4</td>
<td>463</td>
<td>5</td>
<td>34</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>IM</td>
<td>0</td>
<td>714</td>
<td>5</td>
<td>34</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>2</td>
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<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SL</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>280</td>
<td>17</td>
</tr>
<tr>
<td>SR</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>254</td>
</tr>
</tbody>
</table>

Table 2 lists the durations of the determined behaviors of all the four ICR mice. In the table, the diagonal values indicate the correct time duration of each behavior, where the term “correct” implies that the automatically determined result matches that determined manually. All the other values indicate erroneous time durations. The “OT” related data are omitted because it is not a particular behavior.

This table indicates several tendencies for incorrect discrimination by our algorithm.

For rearing, “RE”→“MV”, “RE”→“IM” and “RE”→“HG” are the main misdetections. The main reason for the misdetection of “RE”→“MV” is that the mouse sometimes changed from rearing to other behaviors very quickly which caused the centroid suddenly changed and would be misdetected as “MV”. In some cases, the mouse remained stationary during rearing, and was misdetected as “IM”. In some other cases, the mouse moved the head repetitively during rearing and misdetected as “HG”.

For head grooming, there were several misdetections: “HG”→“OT”, “HG”→“IM”, and “HG”→“SR”. For left-side scratching, “SL”→“OT” and “SL”→“SR” were confused, and misdetection of right-side scratching occurred mainly in case of “SR”→“HG”.

The main reason for such misdetection is that the mouse head cannot always be tracked for segmenting the body parts, particularly when the head overlaps with the body and tail such as when the body is bent or shrink to a large degree. The in-
correctly recognized head positions caused incorrect determination of the motion features. Figure 14 shows the edge images \( C(x, y) \), the silhouette contours \( r_{fl}(x, y) \) in the polar coordinate system, shift-invariant silhouette contours \( \tilde{r}_{fl}(x, y) \) redefined by the incorrectly identified head positions, and the frame-to-frame difference features \( f(x, y) \) in the following misdetection cases: (a) “SL”\( \rightarrow \)SR around \( t = 704^\circ 66^\circ \) in the experiment on ICR1, (b) “SR”\( \rightarrow \)HG around \( t = 576^\circ 82^\circ \) in the experiment on ICR2. The incorrectly identified head positions are compared with the actual head positions in these figures. In panel (a), the identified head actually corresponds to its right ear, and repetitive limb movements around its head were misdetected as right-side scratching, because the head region \( R_h \) involved its actual right-side body region.

5. Conclusions

We described an automatic multiple behavior quantification algorithm for laboratory mice that uses HFR video analysis for quantifying quick and repetitive limb movements at dozens of hertz, such as scratching and grooming. The effectiveness of the authors’ algorithm in animal behavior quantification was demonstrated in experiments involving long-term observations on four ICR mice. In future, the authors aim to increase the practicality of the system and extend it to more complex behaviors and different types of laboratory animals used in animal testing experiments for various medical applications.

References


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