High-throughput phenotyping of mouse grooming

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Abstract

Animal self-grooming behavior is becoming increasingly recognized in neurophenotyping research. Rodent grooming and its complex sequencing are sensitive to various genetic and pharmacological manipulations. However, its phenotyping is usually limited to global endpoints such as frequency of bouts and total grooming duration. In contrast, our study focused on developing a novel, software-driven assay with the ability to quantify the complex sequencing of rodent grooming bouts. Adult male C57BL/6J mice were housed 3-5 animals per cage on a 12:12 h light:dark cycle. The animals were transferred to the experimental room 1 h before testing to ensure proper acclimation. Here, we used custom-upgraded HomeCageScan video-tracking software (Clever Sys. Inc., Reston, VA), to record the grooming behavior of mice in transparent observation cylinders for 5 min. This allowed us not only to perform behavioral quantification of specific grooming patterns (such as paw licking and body/leg grooming) but also analyze the transitions within bouts, revealing significant correlations (P<0.0005-0.02, R=0.51–0.70) with manual observations for total number of transitions and selected specific grooming transitions. In addition, animals were tested for spontaneous and water-induced grooming behavior. Water-induced mice displayed more robust grooming behavior as detected by both manual observers and the custom HomeCageScan software. Both induced and novelty-evoked grooming tests show similar agreement between manual observations and software analysis, validating both models for the further study of rodent grooming behavior. This unique approach is currently being applied to the phenotyping of several mutant strains, including SERT and BDNF knockout mice. Our data suggests that high-throughput automated neurophenotyping of grooming behavior can be developed for biomedical research based on this approach.

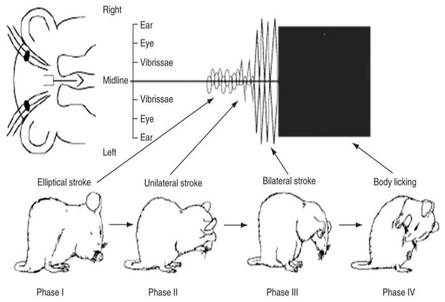

Figure 2. Syntactic grooming chain pattern in mice. Phase I: elliptical strokes tightly around the nose. Phase II: unilateral strokes that reach the mystacial vibrissae to below the eye. Phase III: bilateral strokes ascending high enough to pass over the ears. Phase IV: body licking. Based on Berridge et al., 2006 (adapted from Kalueff et al, 2007, *Nat Protocols* **2**, 2538 – 2544).

Results

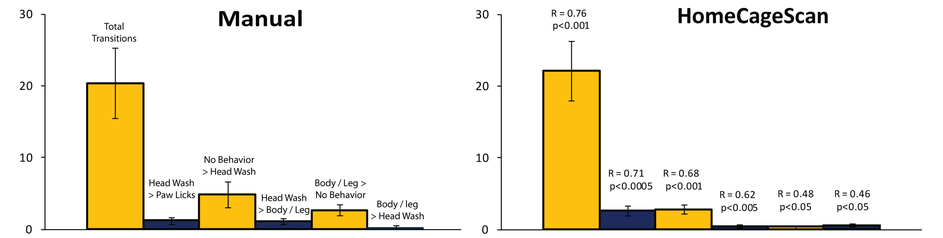

Figure 4. Correlations of manual and automated grooming data obtained in this study. Manual grooming endpoints were compared to CleverSys HomeCageScan data in order to determine the degree of relation between the two methods**.** Significant correlations were found between manual observations and HomeCageScan data using the Spearman rank correlation test.

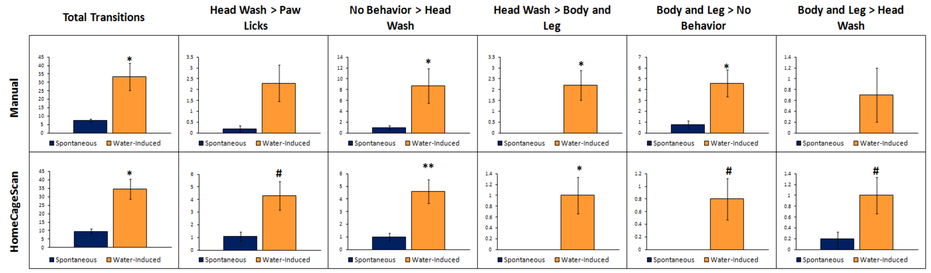

Figure 5. Behavioral differences between patterning (total and specific individual transitions) of spontaneous and water-induced grooming, detected by manual observation (top row) and automated video-tracking software (bottom row). N = 10 in each group, *p≤0.05, **p≤0.005, #p≤0.05-0.1 (trend).

Methods

Housing and acclimation: Adult male C57BL/6J mice were housed at the Tulane Vivarium in cages of 3-5 mice/cage. The subjects had *ad libitum* access to food and water and were placed on a 12-hr light/dark cycle. One hour prior to testing, mice were transferred to the procedure room for acclimation.

Spontaneous (novelty-induced) grooming response: Behavioral testing was performed using a clear novel observation cylinder (15 cm height x 13 cm diameter). Animals were placed in this novel environment and their novelty-induced grooming was video-recorded for 5 min while the experimenters recorded manual data, using the Grooming Analysis Algorithm (GAA) as described previously (see Kalueff and Tuohimaa, 2004; Kalueff et al., 2007 for details).

Figure 1. Typical examples of C57BL/6J mouse grooming observed in this study

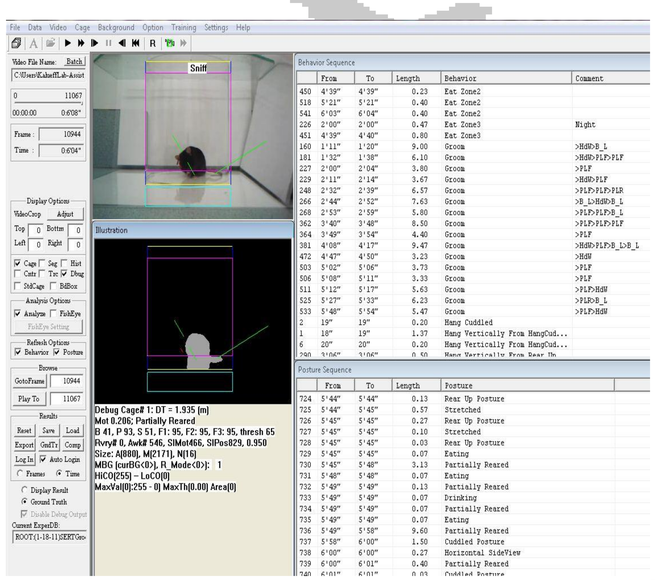

Figure 3. Example of grooming analysis and its output based on HomeCageScan software (CleverSys, Inc). This screenshot illustrates the automatic grooming analysis now possible through HomeCageScan. The original video is shown in the top left of the screen, and directly under that is the analysis screen.

Water-induced grooming response: Subjects were misted with 25°C water 3 times (from 10 cm away) prior to testing. Animals were subsequently placed in the same clear observation cylinder and grooming activity was manually scored and video-recorded for the 5-min testing period.

Video analysis: The recorded videos were analyzed using a custom version of the HomeCageScan software (CleverSys, Inc., Reston, VA). Data was generated for both cumulative measures (total grooming time and number of bouts) and patterning-related endpoints (total transitions between phases and specific transitions) according to the Grooming Analysis Algorithm.

Statistical analyses: Manual observations of grooming behavior were compared to the software-generated data using the Spearman rank correlation test. Spontaneous and water-induced grooming behaviors were compared using the Mann-Whitney U-test (P<0.05).

Discussion

Recent advances in information technology have allowed for improved automated neurophenotyping using various animal models. Here, we have successfully applied the HomeCageScan software (CleverSys, Inc., Reston, VA) for high-throughput grooming research in rodents. As shown in Figure 4, we generated automated data which strikingly correlate with manual observations of mouse grooming. Figures 4-5 demonstrate that the software accurately detects differences not only in overall grooming activity, but also in patterning (transitions) displayed by spontaneously grooming mice (vs. water-induced grooming). The understanding of self-grooming behavior and its correlates will help elucidate the complexities of motor patterning and the neural substrates which drive repetitive behaviors. Given the sensitivity of mouse grooming to various genetic or pharmacological manipulations, ***this new approach allows the researchers to accurately detect changes in both grooming activity and syntax,*** thereby markedly advancing the field of neurophenotyping.

Acknowledgements

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