Automated Maternal Behavior during Early life in Rodents (AMBER) pipeline

Hannah E. Lapp, Melissa G. Salazar, Frances A. Champagne

Department of Psychology

University of Texas at Austin

108 E. Dean Keaton St, Austin, TX 78712

Corresponding author:

Hannah Lapp

Hannah.lapp@austin.utexas.edu

Abstract

Mother-infant interactions during the early postnatal period are critical for infant survival and the scaffolding of infant development. Rodent models are used extensively to understand how these early social experiences influence neurobiology across the lifespan. However, methods for measuring postnatal dam-pup interactions typically involve time-consuming manual scoring, vary widely between research groups, and produce low density data that limits downstream analytical applications. To address these methodological issues, we developed the Automated Maternal Behavior during Early life in Rodents (AMBER) pipeline for quantifying home-cage maternal and mother-pup interactions using open-source machine learning tools. DeepLabCut was used to track key points on rat dams (32 points) and individual pups (9 points per pup) in postnatal day 1-10 video recordings. Pose estimation models reached test errors of 14.39 pixels and 11.81 pixels for dam and pups respectively. Pose estimation data and human-annotated behavior labels from 30 videos were used with Simple Behavioral Analysis (SimBA) to generate behavior classifiers for dam active nursing, passive nursing, nest attendance, licking and grooming, self-directed grooming, eating, and drinking using random forest algorithms. All classifiers had excellent performance, with F_1 scores above .997. A set of 242 videos was used with AMBER and produced behavior measures in the expected range from postnatal 1-10 home-cage videos. This pipeline provides a basis for assessing home-cage dam-pup interactions that reduces experimenter burden while increasing reproducibility, reliability, and detail of data for use in developmental studies without the need for special housing systems or proprietary software.

Introduction

Maternal behavior during early life in mammals ensures offspring survival by supporting the physical needs of the offspring including transfer of nutrients and warmth to young and protection from predators and the environment. Beyond ensuring survival, mother-offspring interactions also guide emotional, social, and cognitive development of offspring. Maternal behaviors provide stimulation to offspring through vestibular, auditory, tactile, and visual modalities during sensitive periods of elevated plasticity early in life. This somatosensory input directs development of pup physiology to have lasting effects on offspring brain and behavior across the lifespan[1–3]. Intergenerational patterns of maternal behavior are observed in several mammalian species, thus effects of these early social experiences can be perpetuated across generations[4].

Factors in the broader ecological environment, such as chemical exposure, distal stressors, resource availability, and social context, can affect offspring development in early life through alterations to maternal offspring-directed behavior[5,6]. Maternal behavior can moderate environmental effects on offspring by buffering the effects of the environment, or increase susceptibility when disruptions to maternal care occur. Maternal effects signal to the infant the conditions of the distal environment and may modify offspring physiology to prepare the offspring for the environment they will encounter later in life [7,8]. Early environmental exposures are also potential contributors to vulnerability for neurodevelopmental disorders, which can be moderated by maternal-offspring interactions[9].

The extensive, enduring effects of mother-offspring interactions and potential for disruption of these interactions by external influences make maternal behavior an important measure for all developmental studies. Laboratory rodent models are a preferred choice for studying the impact of early life exposures on offspring development because of the ability to precisely control environmental conditions, the relatively short lifespan of laboratory rats and mice, overlap in the hormonal and neural mechanisms governing maternal behavior with humans, and ability to examine the molecular physiological outcomes following early life exposures not possible or ethical in human research[10–14]. Like humans, rodent maternal behavior is influenced by the external environment and by offspring physiological and behavioral cues[15–17]. Measures of rodent pup-directed maternal behaviors typically include nest attendance, anogenital and body licking and grooming of pups, nursing (sometimes further specified into blanket, low-arched back, high-arched back, and passive nursing), nest building, and retrieval of pups when they are displaced from the nest[18]. Natural variations of home-cage maternal behaviors in Long-Evans rats are well characterized and variation in these maternal behaviors leads to shifts in offspring developmental trajectories[19–24].

Scoring of home-cage maternal behavior live or through video recordings provides detailed information on dam-pup interactions guiding pup development[25]. However, between-study methodological variation in the acquisition and coding of this data limits comparisons between studies. While time-sampling methods may permit analyses of behavior throughout the circadian cycle, this approach has poor temporal resolution relative to focal observations and prohibits accurate quantification of behavior durations, where point observations about maternal behavior are made throughout the day[26,27]. Frequency, duration, and bout length of maternal behaviors are increasingly used in analyses, particularly measures of “entropy” that are used to

capture consistency of dam behavior patterns[28]. To address these issues, more sophisticated tools are needed to quantify maternal behavior.

Recent advances in machine learning tools for behavior analysis have the capacity to build standardized behavior pipelines for video data to produce high-quality and highly-reproducible measurements[29]. Using open-source tools, we developed the Automated Maternal Behavior during Early life in Rodents (AMBER) pipeline for scoring home-cage maternal behavior. AMBER uses DeepLabCut[30,31] to extract pose estimation data from rat dam and pups from home-cage recordings of standard cages. Side-view recordings permit tracking of key body points on dams and pups, unlike top-down recordings where the dam occludes pups during nest attendance. AMBER then uses Simple Behavior Analysis[32] (SimBA) to run behavior classifiers for seven maternal behaviors: nest attendance, licking and grooming, active nursing, passive nursing, self-directed grooming, dam eating, and dam drinking. We applied the AMBER pipeline to a set of 242 videos from postnatal day (P) 1-10 to evaluate maternal phenotype. All scripts and models used in the pipeline are publicly available (https://github.com/lapphe/AMBER-pipeline; https://osf.io/e3dyc/).

Results

Video recording

Home cage behavior was recorded from Long-Evans dams and litters on postnatal day (P) 1-14 with Raspberry Pi 3B+ minicomputers equipped with Raspberry Pi Module 1 NoIR cameras. Full Raspberry Pi recording setup instructions and recording scripts are available at https://github.com/lapphe/raspberry_rat. One hour and 24-hour video recordings captured video during the light and dark phase under infrared LED lights.

Dam pose estimation model

Pose estimation for dams was achieved using single animal DeepLabCut[30]. 4,710 frames were extracted from 255 videos. Thirty-two dam body points were labeled providing adequate coverage of the dam regardless of partial body occlusion or body orientation relative to the camera (**Figure 1B**). Two percent of labeled frames were used for a test set for model evaluation and the remaining 98% of labeled frames were included in the training set. The dam model was trained for 650,000 iterations saving a snapshot every 50,000 iterations using ResNet-50 with a batch size of 8. Model performance was evaluated at all snapshots and loss was calculated every 1000 iterations (**Figure 2A**). Snapshot 10 (550,000 iterations) had the best performance on test frames with an average error of 14.39 pixels on test frames and 6.36 pixels on training frames after filtering to only points with a likelihood cutoff threshold above 0.5 (**Figure 2B**). The width of one dam ear was approximately 13.9-44.5 pixels depending on dam location in the cage. Visual inspection of labeled held-out videos confirmed model performance.

Pup pose estimation model

The pup pose estimation model was developed using multi-animal DeepLabCut[31]. 1,712 frames from 238 videos were extracted using k-means clustering and uniform distribution. Nine pup points from the nose to tail base were labeled on each pup visible (**Figure 1C**). Two percent of labeled frames were reserved for model evaluation and the remaining 98% of labeled frames

were included in the training set. The pup model was trained for 200,000 iterations saving a model snapshot every 5,000 iterations using DLCRNet_ms5 with batch size of 8. Model loss was calculated every 1000 iterations (**Figure 2C).** Pup pose estimation model performance was evaluated at all snapshots (**Figure 2D**). The final snapshot had the best performance, with an average error of 4.67 pixels on test frames and 11.81 pixels on training frames after filtering to points with a likelihood cutoff threshold above 0.5. Root mean square error for individual pups and each body point was similar (**Supplemental Figure 1**). Visual inspection of labeled held-out videos confirmed accurate tracking of individual pup body parts for pup detections.

Pose estimation data postprocessing

Pups are often in a huddle mass in the nest in the home-cage, with many pups fully or partially occluded by other pups and nesting material at any given time. This occurrence presents a challenge for assignment of pup body part detections to individual pups during tracklet creation and tracklet stitching across frames during the second half of the multi-animal DeepLabCut workflow. Although pup detections achieved good performance, we observed substantial loss of pup points after individual assembly, where only pups with a majority of tracked body points visible had reliable tracking. A primary goal of tracking pups for this pipeline is to identify nest location, so the group of pups can be treated as a unit rather than as individuals. Therefore, pup detections, obtained by running the DeepLabCut function analyze_videos with auto_track= false, rather than individual tracks, were used for pup pose estimation. A custom python script (pheno_pickle_raw.py) was used to convert the pup detections pickle file to a csv file containing pup key point detections. Thus, raw detection key points assigned to individual pups after conversion to csv do not necessarily belong to the assigned pup.

After pickle file conversion to csv, unfiltered dam and pup pose estimation files were joined by frame number using a custom script (join_dam_pup.py). Column headers were reformatted to match the expected input by SimBA for single animal projects.

Behavior classifier project set up

Simple Behavior Analysis (SimBA) was used to generate seven behavior classifiers from pose estimation data from a subset of videos using the standard workflow except where noted below (https://github.com/sgoldenlab/simba)[32]. Since SimBA expects the same body point configurations for each individual in multi-animal projects, we used a single animal SimBA project configuration with user-defined body points consisting of all dam and pup key points. The width of the wire cage top at the lowest point was used to define pixels per mm during the Video Settings (**Supplemental Figure 3**). The outlier correction step of SimBA was skipped because this step relies on body-length distance across all frames to perform these calculations, which is influenced by the dramatic differences in body length when the dam is near the front versus back of the cage.

Classifier feature extraction

SimBA behavior classifiers train on features derived from pose estimation data. Dam and pup features were extracted from the pose estimation data using a custom script

(amber_feature_extraction.py) to better separate dam-specific and pup-specific features (**Supplemental Table 1**). Features were derived from pup pose estimation data (11 features), dam pose estimation data (200 features) or both dam and pup data (6 features). Features can also be broken down into categories: 1) coordinate information (e.g. centroid coordinates, point probability scores), 2) areas (e.g. convex hull area of all pup points, convex hull of dam head points), 3) distances (e.g. length of dam body, distance from dam to pup centroids), and 4) movement (e.g. movement of individual body points). Features can also be divided by type: raw value (e.g. adjusted centroid x and y coordinate), sums (e.g. sum of all pup point probabilities), and means (e.g. mean movement of all dam back points). Finally, features can be described in terms of the time frame the feature is derived from: information within a frame, between frames (.03s for 30 fps recording), 0.1s, 1s, or 2s rolling windows.

Random forest behavior classifiers

Classifier training videos were carefully annotated for seven maternal behaviors using BORIS[33] then imported into SimBA. Behavior definitions are provided in **Figure 3**[16,17]. A total of 2,462,599 frames from 30 videos were annotated for nest attendance, active nursing, passive nursing, and licking and grooming and 1,837,760 frames were annotated from 25 videos for self-directed grooming, eating, and drinking (**Supplemental Table 1**). Frequency of behaviors ranged from 3.6% (passive nursing) to 60.4% of frames (nest attendance). Random forest models were run with the following hyperparameters: n_estimations = 2000, RF_criterion = entropy, RF_max_features= sqrt, RF_min_sample_leaf = 1, test size= 20%. No sampling adjustment was used, except for the passive nursing classifier where Random undersampling was used at a ratio of 16:1 to account for the low frequency of this behavior.

Behavioral classifier evaluation

Behavior classifiers were evaluated by calculating the precision (fraction of true positives among all frames scored as positive), recall (fraction of true positives retrieved out of all true positives in data set), and F1 scores (harmonic mean of precision and recall) for all models (**Supplemental Table 2**). All behavior classifiers obtained good accuracy at or above 0.995. Discrimination thresholds, or probability above which a behavior is classified as present, were determined using precision-recall curves and visually inspecting behavior probabilities from a held-out video. Precision, recall and F1 scores remained high across a wide range of discrimination thresholds (**Figure 4**). Validation videos confirmed accuracy of behaviors classifiers using these discrimination thresholds.

Post-hoc explanability metrics for behavior classifiers

Explanability metrics offer interpretable descriptions for how models decisions are made from feature values[34]. Feature importance permutations provide an estimation of information loss when the feature is replaced with randomly shuffled values from the same distribution as the original feature data. Feature importance permutations were calculated for each behavior classifier with eli5 python library in SimBA. The range of feature importance estimates varied between models, with a narrower distribution for drinking, passive nursing, and nest attendance, and wider distributions for active nursing, eating, and self-directed grooming (**Supplemental**

Figure 3). Relative importance of each feature within models was determined by ranking features from most important (rank 1) to least important (rank 217) based on feature importance score for between-model comparison. The average rank for each feature category was calculated for heatmap visualization (**Figure 5**). Dam-pup distance features are the most important feature category (lowest rank) for nest attendance, licking and grooming, and active nursing. Dam movement is the least important average feature (highest rank) for all models except the drinking classifier. Dam head and dam body centroid coordinates, movement of dam back points, and convex hull areas of the dam head and body are among the top five features with the highest importance scores for several classifiers.

Shapely Additive Explanations (SHAP) is another explanability metric that uses a game theoretic approach that can be applied to tree-based machine learning models to allocate the contributions of individual features to the overall final behavior probability in a frame based on magnitude of feature attributions[34,35]. SHAP analysis was run in SimBA on 100 random frames with behavior present and 100 random frames with behavior absent for each model to calculate individual feature contributions to overall frame behavior probability (https://github.com/slundberg/shap). **Figure 6A** shows the top five features with the largest absolute SHAP scores for each behavior classifier, where the solid black line is the base rate for the behavior (probability of a given frame containing the behavior by chance), each individual point reflects the change in behavior probability relative to the base rate (SHAP score) for that feature for one frame, and the color of the point reflects the z-score of the actual feature value for that frame. Consequently, the relationship between behavior probability shift and actual feature value can be deduced as positively or negatively associated. For on-nest dam behavior classifiers, features with high SHAP scores are those containing information about dam y coordinates, dam areas, and dam-pup distances. Top features of other classifiers were more varied and include dam x and y coordinate, dam movement information, and body-length information. Sum of SHAP score for all features by feature categories (**Figure 6B**) show that dam movement features did not have a substantial impact on nest attendance, but have a moderate influence on increasing behavior probabilities in remaining models and had a particularly large effect on licking and grooming and active nursing. Pup coordinate and area features had little effect on behavior probabilities. The sum of SHAP scores time frame show that feature information within frames generally had the largest effect on behavior probabilities, followed by 2s, 1s, 0.1s, and .03s windows in that order (**Supplemental Figure 4**).

Pipeline validation for maternal phenotype

242 videos of one-hour home cage recordings taken on P1-10 from 49 dams were used in the AMBER pipeline workflow as shown in **Figure 1A**. Thresholds used for behavior classifiers are noted in **Supplemental Table 2**. Total duration, percent time, bout number, and mean bout duration were calculated in SimBA for each behavior for each video (**Figure 7** and **Supplemental Figure 5**). The mean percent time engaging in the behavior across all videos was 38.7% for nest attendance (min = .05%, max = 100%, median = 31.5%), 8.6% for licking and grooming (min=0, max = 32%, median = 7.8%), 14.4% for active nursing (min = 0, max = 77.2%, median = 7.8%), 2% for passive nursing (min = 0, max = 55%, median = <1%), 4.5% for self-directed grooming (min = 0, max = 54.3%, median = 1.2%), <1% for eating (min = 0, max =

19.6%, median = <1%), and 4.5% for drinking (min = 0, max = 53.4%, median = <1%). Change in behavior durations over time was analyzed using linear mixed models in R with the lme4 package with litter ID included as a random effect[36]. Nest attendance (β = -187.13, t = -10.45, p< .001), licking and grooming (β = -19.420, t = -4.257, p< .001), active nursing (β = -100.15, t = -8.231, p< .001), passive nursing (β = -13.32, t = -2.967, p=.003) significantly decreased with litter age. Self-directed grooming (β = 26.306, t = 4.725, p<.001), eating (β = 2.660, t = 2.037, p=.042), and drinking (β = 27.375, t = 4.825, p<.001) significantly increased with litter age (**Figure 7**).

Discussion

Though continuous home-cage monitoring is an optimal approach to assess dam-pup interactions in a laboratory setting, the implementation of this approach has been limited. The AMBER pipeline automates scoring of rodent dam-pup home-cage video recordings to produce frame-level annotations of seven maternal behaviors with high accuracy. AMBER eliminates the time-burden, reproducibility concerns, inter-rater reliability obstacles, and experimenter bias drawbacks of manual scoring. The pipeline uses open-source software, standard rat housing equipment, and does not require any specialized recording equipment or animal identification markers. When paired with automated recording equipment, home-cage behavior can be collected from an entire cohort of animals simultaneously for long time periods over the first two postnatal weeks while avoiding the effects of experimenter presence or bias on behavior.

Model explanability metrics shed light on the “black-box” of behavioral classification by providing articulated descriptions of how features influence model performance that allows users to critique model construct validity and compare different models beyond model performance[34]. Feature importance permutation results showed low importance of dam movement features. The majority of features comprising the dam movement category are the movement of individual dam body points, so this may suggest that this information is less informative or reliable for behavior classifier predictions than features that use information from multiple body points. The drinking classifier feature importance ranks contrasted the other models, relying heavily on pup areas and dam movement and less on other feature categories on average. SHAP analysis revealed several intuitive relationships between dam pup-directed behaviors and change in behavior probability in our classifiers: 1) the nest is located at the front of the cage, so the y coordinate of dam body points will increase as she moves lower down in the frame toward the nest; 2) the convex hull areas of the dam will get larger as she moves toward the nest (closer to the camera) at the front of the cage; 3) dam-pup Euclidian distances will decrease when the dam is interacting with pups and will increase when the dam is off-nest; 4) dam movement features are not informative for nest attendance since the dam can be very still (as during nursing bouts) or highly active (as during licking and grooming) while on the nest, but movement information is informative for all other classifiers that can be operationally defined by specific body movements (e.g. licking and grooming) or lack of movement (e.g. active nursing). We did not account for water or food location in our training videos in attempt to build classifiers that are agnostic to food and water location. However, rat dams typically choose to build their nest at the end of the cage opposite the water bottle spout direction, putting the water on the right side of the frame and food on the left. This was the case in 21 of the 25 videos included in the training set for eating

and drinking classifiers. This pattern of food and water location explain the high SHAP scores for some dam x coordinate features and the opposite effect of dam x coordinate data on eating compared to drinking probabilities (**Figure 6A**). The interpretation of the dam x coordinate features with high SHAP scores for passive nursing and self-directed grooming is less clear, and may reflect unintentional bias in dam location in the training data that may be corrected with inclusion of additional diverse behavior examples in the training set.

The validation set of recordings show that AMBER-scored videos produce expected patterns of maternal behaviors over the first ten postnatal days. The duration of pup-directed behaviors was high on P1 and steadily declined over the first week while the durations of dam self-care behaviors (self-directed grooming, eating, drinking) increased. This is consistent with previous work from manually-coded behavior reporting declines in dam-pup contact and licking and grooming from P1-10[37]. Licking and grooming and nest attendance were relatively normally distributed, with the range of percent of time for licking and grooming similar to frequency observed in time-sampling studies for Long-Evans rats[37]. Active nursing was not normally distributed, although this difference may be attributed to the one-hour observation during early dark phase for the present study versus sample observations throughout the dark and light cycle used in previous work[38]. The number of bouts for behaviors was very high in a few videos (**Supplementary Figure 5**) and mean bout duration lower than expected. This is explained by the frame-level behavior measurements, where one frame labeled with the behavior presence (or absence) is sufficient to determine a "bout". Smoothing methods such as employing a larger minimum bout duration or Kleinberg burst detection can be applied directly in SimBA to smooth the data.

Using the AMBER pipeline as presented in **Figure 1A**, users can get frame-level resolution of seven maternal behaviors. However, the individual components of AMBER (dam pose estimation, pup pose estimation, and behavior classification) can also be used for other applications. First, dam pose estimation and pup pose estimation models may also be used separately in other contexts to track adult rats and pups in any side-view recording. Many published pose estimation models for rodents are for top-down or bottom-up recording which are not always possible or preferred. Second, pose estimation data may be used with compatible software to perform unsupervised behavior clustering[39–41]. Third, specific features extracted from the pose estimation data may be informative in concert with behavior annotations. For example, the convex hull area of pups, dam centroid-pup centroid distance, or amount of movement of the dam while on the nest may be of interest. These data are calculated during the AMBER pipeline and are readily available for further analysis. Finally, the small size of neonatal pups and large number of pups in the litter makes manual behavior scoring for pups very difficult. While the primary purpose of the pup pose estimation model in the AMBER pipeline is to determine pup and nest location and dam-pup distances, pup pose estimation data could be used separately to evaluate pup behavior in relation to dam behavior.

AMBER is a substantial improvement over manual scoring methods for dam-pup home cage behavior, but also has some notable limitations. Classifier performance for pup-directed behaviors may be compromised in recordings where pups are occluded for the duration of the video since pup coordinate information and dam-pup distances are important features for several classifiers. This shortcoming could be circumvented by manually adding nest location

information in place of pup tracking. Furthermore, the behavior classifiers presented here are trained on tracking information from side-view recordings and will not generalize to top view recordings. We chose side view recordings to allow for better pup tracking and to eliminate the need for any specialized home-cage equipment as many standard home cages contain food and water in the cage lid. Finally, the pose estimation models at present are optimized for detecting key points in Long-Evans rats and are unlikely to generalize well to other rodent species that are visually different without training on additional frames. Likewise, differences in camera angle, bedding material, enrichment objects, cage layouts, or lighting in recordings compared to the training videos may interfere model transferability, requiring some additional labeled frames and pose estimation model retraining[42]. We are currently expanding these model training sets to include frames from videos of Sprague-Dawley rats, C57/Bl6 mice, and CD1 mice in different home cages to make the pose estimation models more robust and able to perform well for a wider variety of rodent developmental studies. Despite these current limitations, the AMBER pipeline is a significant step forward for improving analysis of home cage dam-pup interactions to provide standardized, detailed behavioral data likely to yield new insights in developmental studies.

Materials and methods

Animal husbandry and breeding

All animal protocols were approved by the IACUC at the University of Texas at Austin, were performed in accordance with IACUC guidelines and regulations, and are reported in accordance with the ARRIVE guidelines. Animals were housed in polycarbonate cages (19” × 10.5” × 8”) with standard wire tops, Aspen bedding (Nepco), and were kept on a 12:12 h light cycle (lights off at 10 am EST). All animals were fed standard chow (Lab diet 5LL2) and water *ab libitum* through bottles held at approximately a 45-degree angle in the wire tops. Eighty-eight adult P60–70 Long-Evans females and 35 adult Long-Evans males were purchased from Charles River Labs and acclimated to the vivarium for at least two weeks before breeding. During breeding, P75–85 females were screened daily for receptive behavior and housed with a breeder male overnight on the day lordosis was observed. All dams were socially housed throughout pregnancy until they were separated into individual cages a few days before giving birth. Day of birth was considered P0.

Video recording

Home cage behavior recording was conducted with Raspberry Pi 3B+ minicomputers running Debian bullseye with the Raspberry Pi Desktop and equipped with Raspberry Pi Module 1 NoIR cameras. One Raspberry Pi was placed perpendicular to the short end of the cage and closest to the nest location for each cage. Pis were held in place using phone mounts attached to magic arms clamped to the rack. Raspberry Pis were programmed to record for one hour starting an hour after lights-off at 30 fps in greyscale at 1280 X 780 or 920 X 550 resolution on postnatal day (P) 0-13. Two infrared LED strip lights (940nm; LED Lights World) were attached to the bottom of the wire shelf above the cages and set to turn on and off for the recording automatically with a digital timer. Another 156 videos in were taken to capture 24-hour recordings at 2 fps and 920 X 550 resolution to capture video footage during both the light and

dark phases. Only videos recorded at 30fps were used to build behavior classifiers. Raspberry Pis were headless and accessed remotely in order to prevent disruption to home cage behavior by experimenter presence before or during the recordings. Following video recording, videos were automatically converted to mp4 format with MP4Box and uploaded to cloud storage. In the event that the dam moved the location of the nest to the opposite end of the cage, the camera side was also switched at the first opportunity. Full Raspberry Pi recording setup instructions and recording scripts are available at https://github.com/lapphe/raspberry_rat.

Pose estimation models

Pose estimation for dams was achieved using single animal DeepLabCut[30]. A total of 4,710 frames were extracted from 255 videos. Thirty-two dam body points were selected from 60 candidate body points for labeling based on the user ability to label key points consistently and to provide adequate coverage of the dam regardless of partial body occlusions or orientation relative to the camera (**Figure 1B**). Only body points that were visible in the frame were labeled. For example, if only the left side of the body was visible, points on the right arm, leg, and ventrum were not labeled. Two percent of labeled frames were used for a test set for model evaluation. The dam model was trained for 650,000 iterations using ResNet-50 with a batch size of 8.

The pup pose estimation model was developed using multi-animal DeepLabCut[31]. 1712 frames from 238 videos were extracted using k-means clustering and uniform distribution. Nine pup points from the nose to tail base were labeled on each pup visible in the frame (**Figure 1C**). Two percent of labeled frames were used for a test set. The pup model was trained for 200,000 iterations using DLCRNet_ms5 with batch size of 8. Pup detections, rather than individual tracks, obtained by running the DeepLabCut function analyze_videos with auto_track= false, were used for pup pose estimation. A custom python script (PhenoPickleRaw.py) was used to convert the pup detections pickle file (ending in “full.pickle”) to a csv file containing the pup key point detections. Unfiltered dam and pup pose estimation files were joined by frame number using a custom R script (join_dam_pup.py). Column headers were reformatted to match the expected input by SimBA for single animal DeepLabCut pose estimation.

Behavior classifier development

A SimBA single animal project configuration with a user-defined body points consisting of all dam and pup key points was used for creating behavior classifiers. The width of the wire cage top at the lowest point corresponds to approximately the center of the long side of the cage and was used to define pixels per mm during the Video Settings (see **Supplemental Figure 3**). Because of the side camera view, the actual pixel/mm distance will change based on the dam and pup’s location in the cage, but setting this distance helps account for differences in cage distance from the camera and frame resolution between recordings. The outlier correction step of SimBA was skipped.

Dam and pup features were extracted from the pose estimation data using a custom script (pheno_extract_script_10_6.py) Classifier training videos were carefully annotated for seven maternal behaviors using BORIS[33] then imported into SimBA. A total of 2,462,599 frames from 30 videos were annotated for nest attendance, active nursing, passive nursing, and licking and

grooming and 1,837,760 frames were annotated from 25 videos for self-directed grooming, eating, and drinking (**Supplemental Table 2**). All Random forest models were run with the following hyperparameters: n_estimations = 2000, RF_criterion = entropy, RF_max_features= sqrt, RF_min_sample_leaf = 1, test size= 20%. No sampling adjustment was used, except for the passive nursing classifier where Random undersampling was used at a ratio of 16:1 to account for the low frequency of this behavior.

Explanability metrics for behavior classifiers

Feature importance permutations were calculated for each behavior classifier in SimBA. SHAP analysis was run in SimBA on 100 random frames with behavior present and 100 random frames with behavior absent for each model. Full results files for feature importance permutations and SHAP analysis are available on our OSF repository: https://osf.io/e3dyc/

Pipeline validation for maternal phenotype

242 videos of one-hour home cage recordings taken beginning one hour after lights off on P1-10 from 49 dams were used in the AMBER pipeline workflow as shown in **Figure 1A**. Thresholds used for behavior classifiers are noted in **Supplemental Table 2**. Total duration, percent time, bout number, and mean bout duration were calculated in SimBA for each behavior for each video (**Figure 7** and **Supplemental Figure 5**). Change in behavior durations over time were analyzed using linear mixed models in R with the lme4 package with litter ID included as a random effect[36].

Computer hardware and software

All models were trained on a Dell Precision 7920 Tower with a Dual Intel Xeon Gold 5122 3.6GHz processor, 64GB RAM, Windows 10 operating system, and a NVIDIA Quadro P5000 video card. Pose estimation models were trained using DeepLabCut version 2.3. Behavioral classifiers were generated using SimBA version 1.31.2. Python and R were used for pickle file conversion and pose estimation data joining.

Data availability

Pose estimation models and scripts are available on: https://github.com/lapphe/AMBER-pipeline. Behavior classifier models are available on our OSF repository: https://osf.io/e3dyc/. Instructions for implementing the AMBER pipeline are available at: https://github.com/lapphe/AMBER-pipeline/wiki. Please contact the corresponding author with additional inquiries.

Author contributions

HL collected video recordings, developed the pipeline, analyzed the validation video set, created the figures and tables, and wrote the main text. MS collected video recordings, annotated behavior videos, and edited the text. FC conceived and designed the study, supervised the project, and revised the text. All authors approved the manuscript.

Competing interests

The authors declare no competing interests.

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Figure 1. Project pipeline and key point tracking. A) AMBER pipeline overview. After video recording, pose estimation is accomplished using trained networks to detect dam and pup key points with single animal DeepLabCut and multianimal DeepLabCut respectively. Next, coordinate pose estimation data for dams and pups is joined in one csv file. SimBA behavioral classifiers trained to detect seven maternal behaviors are run on the pose estimation data to produce frame-level behavior annotations. B) Dam pose estimation key points. The dam pose estimation model is trained to detect 32 key points on dams. C) Pup pose estimation points. The pup multi-animal model is trained to identify nine key points on each individual pup

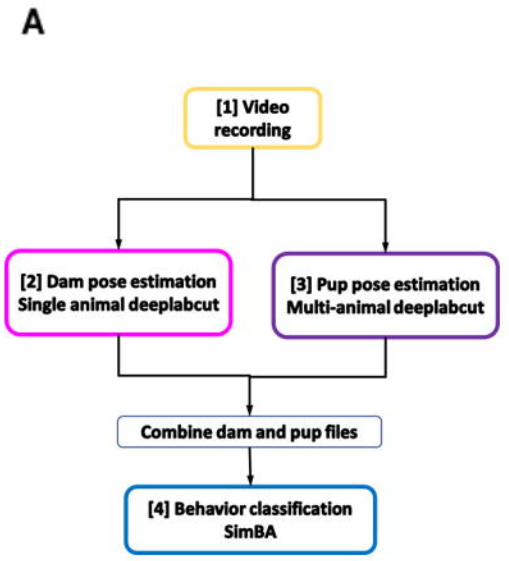
Figure 2. A) Dam pose estimation and C) Pup pose estimation model training statistics. Loss decreased with each training iteration. B) Dam and D) Pup pose estimation model snapshot evaluations. RMSE plotted for training and test sets with and without a probability cutoff of 0.5 at each snapshot during model training.

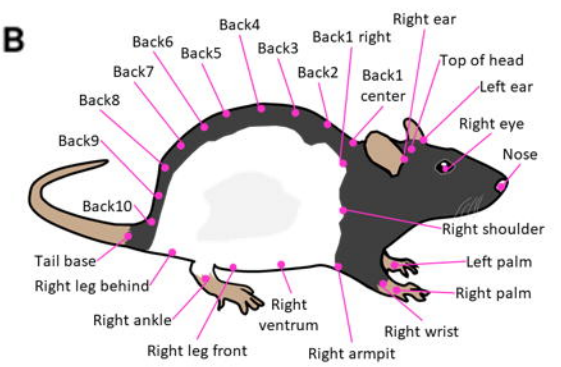
Figure 3. Behavior descriptions for each of the seven maternal behaviors annotated to train the SimBA behavioral classifiers.

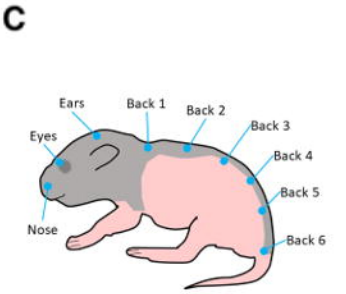
Figure 4. Accuracy-discrimination threshold curves for SimBA classifiers. Precision, recall and F1 scores at different discrimination thresholds are shown for all behavior classifiers.

Figure 5. Relative importance of feature categories as determined by feature importance permutations. All features were ranked from highest (1) to lowest (217) based on mean feature importance score for each behavior classifier. The title color corresponds to the average rank of all features belonging to each category. Dark blue tiles are indicative of higher feature importance (lower rank) and yellow tiles are indicative of less important feature categories (higher rank).

Figure 6. SHAP results. A) Top five features with highest SHAP scores for all models. SHAP scores with the largest average absolute value across all tested frames are shown for each behavior classifier. Each point denotes the SHAP score for one of the 100 frames included in SHAP analysis. The solid black line shows the base rate for each classifier, or the probability that the behavior occurs given the frequency of behavior before consideration of feature information. Base rates vary by classifier and are shown in Supplemental Table 2. SHAP values greater than the base rate indicate positive SHAP scores, increasing the behavior probability. SHAP scores below the black line indicate scores that reduce the behavior probability. Each point is colored by the z-scored value of the feature for that specific frame, indicating the relationship between feature values and change in behavior probabilities. B) Sum of SHAP scores of all features in each feature category for all classifiers. Positive SHAP scores indicate the magnitude of the feature influence on increasing the behavior probability when the behavior is present and negative SHAP values indicate influence on reducing the behavior probability in frames where the behavior is absent. 0 represents the base rate of behavior in the training dataset.





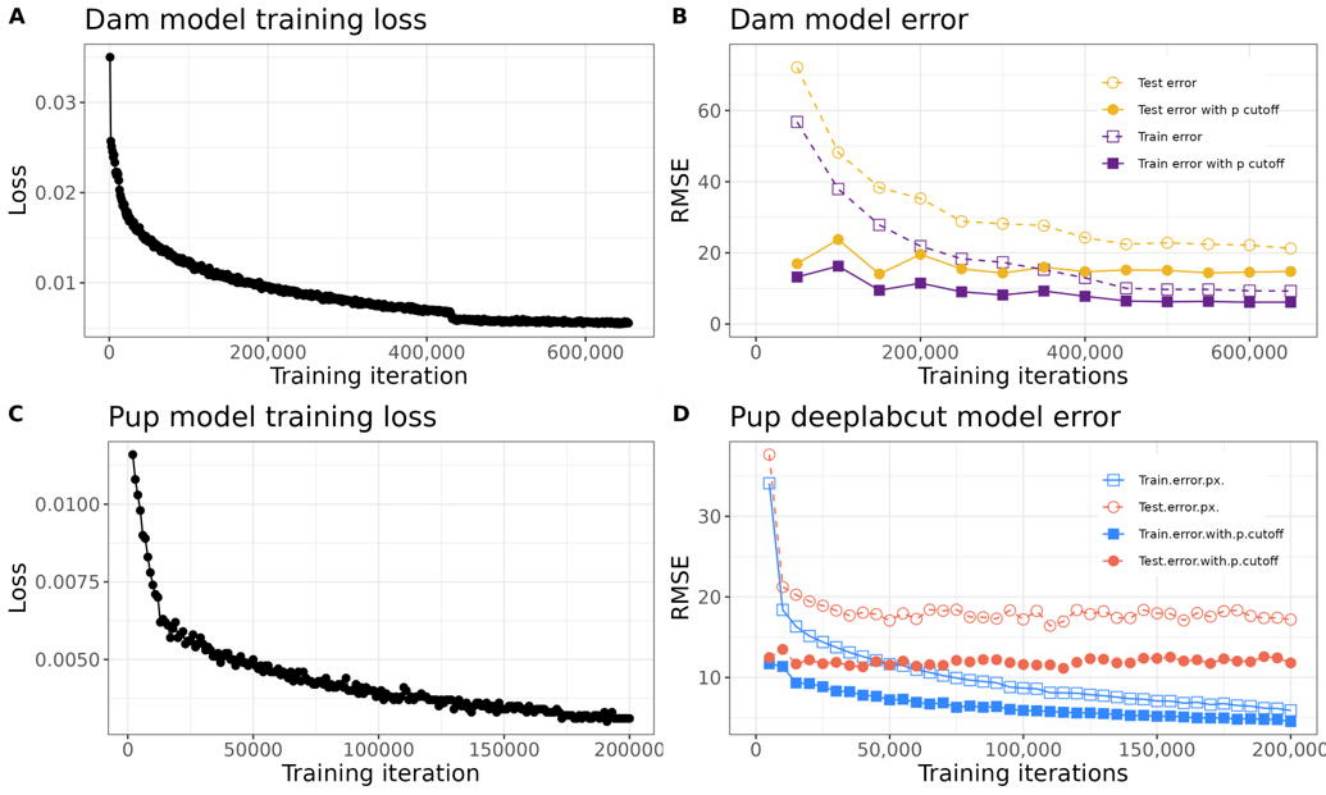


All dam labels

Nose	Back 6	Left/right leg front
Left/right ear	Back 7	Left/right leg back
Left/right eye	Back 8	Left/right ankle
Top of head	Back 9	Left/right ventrum
Back1 left/right	Back 10	Center ventrum
Back1 center	Tail base	Between arms
Back 2	Left/right armpit	Between legs
Back 3	Left/right shoulder	
Back 4	Left/right wrist	
Back 5	Left/right palm	

All pup labels

Nose
Eyes
Ears
Back1 left/right
Back 2
Back 3
Back 4
Back 5
Back 6


A
Dam model training loss
Loss
0.03
0.02
0.01
0
200,000
400,000
600,000
Training iteration
B
Dam model error
RMSE
60
40
20
0
Test error
Test error with p cutoff
Train error
Train error with p cutoff
0
200,000
400,000
600,000
Training iterations
C
Pup model training loss
Loss
0.0100
0.0075
0.0050
0
50000
100000
150000
200000
Training iteration
D
Pup deeplabcut model error
RMSE
30
20
10
Train.error.px.
Test.error.px.
Train.error.with.p.cutoff
Test.error.with.p.cutoff
0
50,000
100,000
150,000
200,000
Training iterations

Nest attendance: A portion of the dam's body is in the nest* and in contact with the pups. Nest attendance begins when the majority of the dam's head (up to her eyes) or her back side is in the nest and ends when she is no longer touching the pups. If the dam is above the nest and is not interacting with or in contact with the pups, then nest attendance is not scored.
Minimum duration: 1 second
**Nest is defined as at least half of the pups grouped together in one area of the cage*

Licking/grooming: The dam is licking and grooming a pup's body or anogenital region with contact between her nose and mouth and the pup. If the pup the dam is interacting with is occluded, licking and grooming is scored when her head is pointed down and is moving in a rhythmic motion consistent with licking and grooming (Moore, 1996).
Minimum duration: licking and grooming one pup for at least 1 second or multiple pups for at least 2 seconds cumulatively

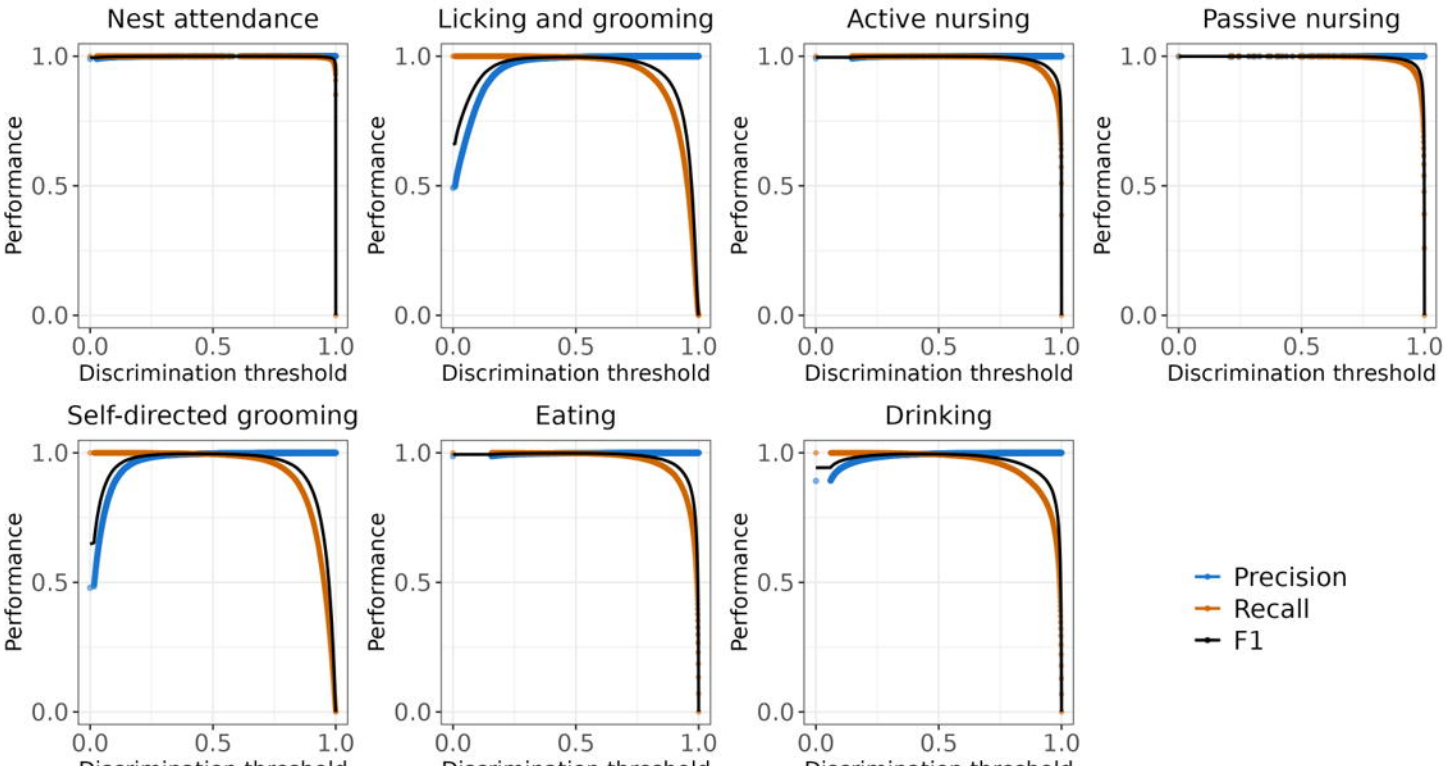
Active nursing: Dam is crouched above the pups supporting her own weight while nursing. Typically, the dam's fore- and hind-limbs rigid and splayed to the side, her back will be arched, her head may be depressed, and she is usully immobile. Some or all pups are attached to the dam's nipple (Stern & Johnson, 1989). Includes high arch, low arch, and blanket nursing and may co-occur with licking and grooming.
Minimum duration: 3 seconds

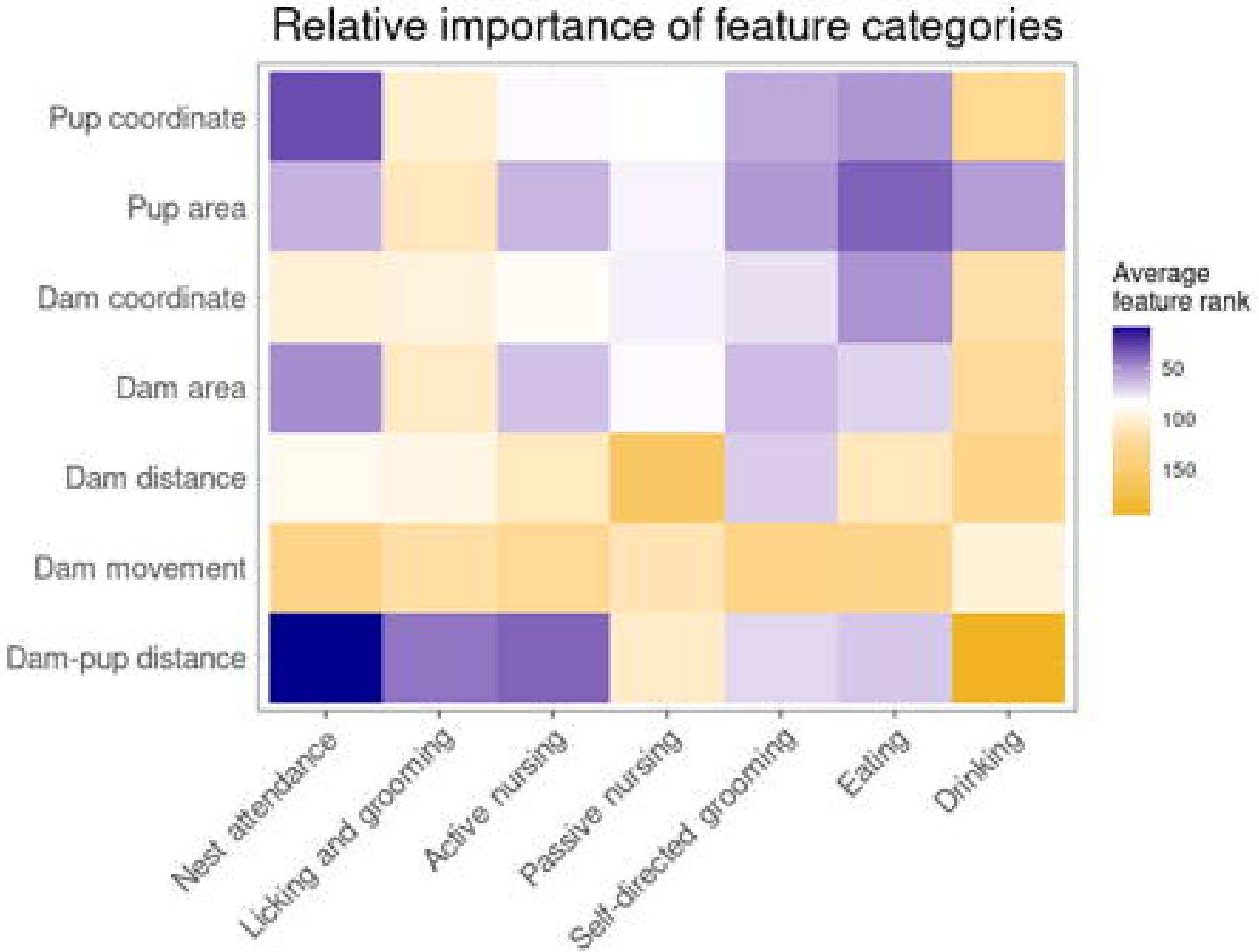
Passive nursing: Dam is on her side and is not supporting herself with her limbs while pups are seen attached to the her nipples. The left or right fore- and hind-limbs may be in the air. The dam is typically immobile during passive nursing.
Minimum duration: 3 seconds

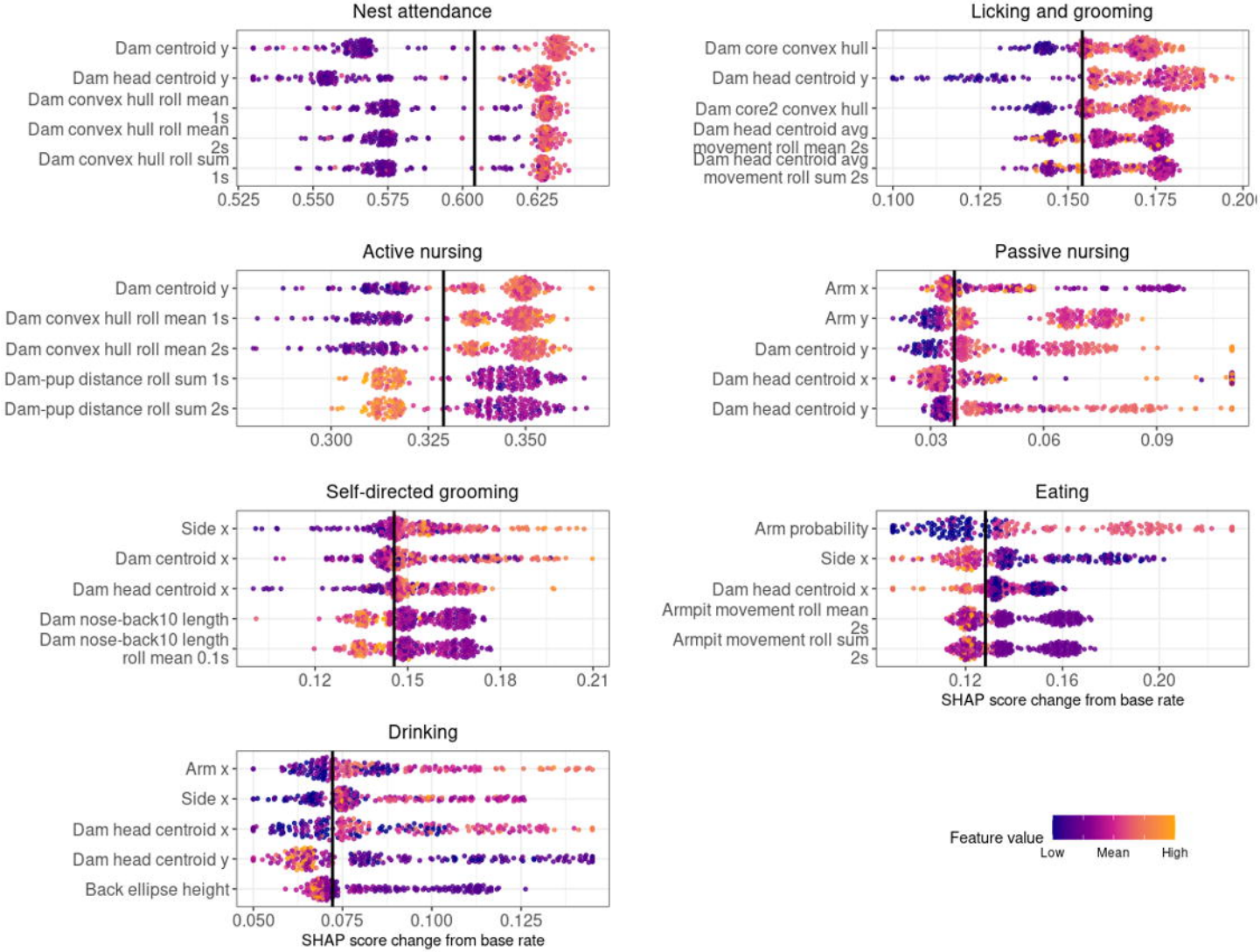
Self-directed grooming: Dam is stroking her body or face with her hands in elliptical, unilateral, or bilateral strokes or is licking her body. These behaviors are usually repetitive and stereotyped. Self-directed grooming may occur during nest attendance and is often observed in alteration with pup-directed licking and grooming bouts during nest attendance.
Minimum bout duration: 1 second

Eating: Dam is eating food available through the wire cage top. Eating is scored when she is actively retrieving food from the top and when she is eating from a piece of food held in her hands.
Minimum bout duration: 1 second

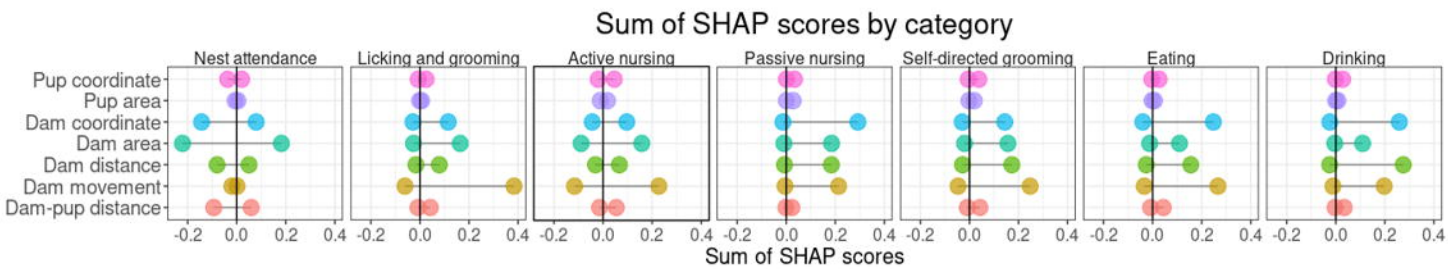
Drinking: Dam is licking water from the water bottle available through the wire cage top. The water bottle spout may be towards or away from the camera. Behavior begins when her mouth comes in contact with the spout and ends when she breaks contact.
Minimum bout duration: 1 second

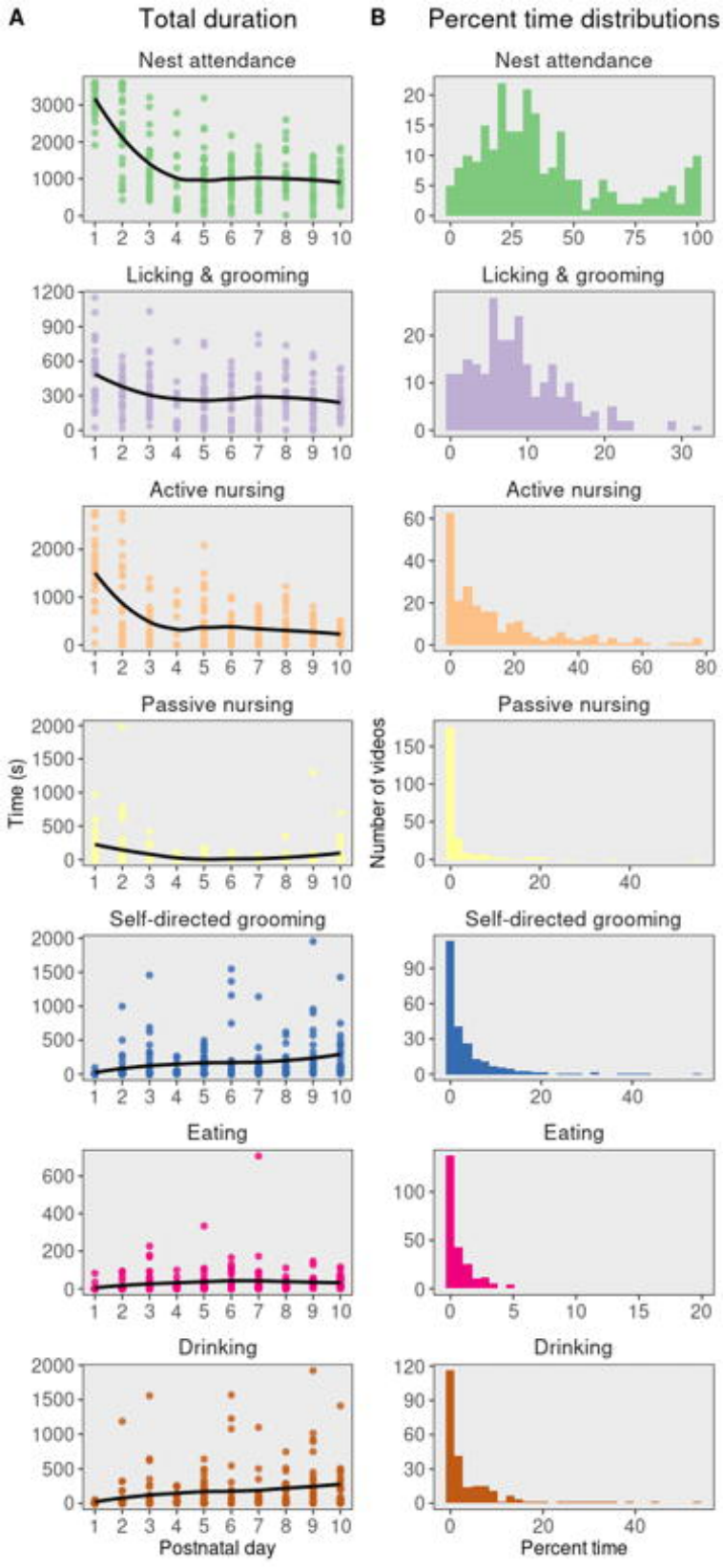

Nest attendance
Licking and grooming
Active nursing
Passive nursing
Self-directed grooming
Eating
Drinking
Performance
Discrimination threshold
0.0
0.5
1.0
Precision
Recall
F1


Relative importance of feature categories
Pup coordinate
Pup area
Dam coordinate
Dam area
Dam distance
Dam movement
Dam-pup distance
Nest attendance
Licking and grooming
Active nursing
Passive nursing
Self-directed grooming
Eating
Drinking
Average feature rank
50
100
150


Nest attendance
Dam centroid y
Dam head centroid y
Dam convex hull roll mean 1s
Dam convex hull roll mean 2s
Dam convex hull roll sum 1s
0.525
0.550
0.575
0.600
0.625
Licking and grooming
Dam core convex hull
Dam head centroid y
Dam core2 convex hull
Dam head centroid avg movement roll mean 2s
Dam head centroid avg movement roll sum 2s
0.100
0.125
0.150
0.175
Active nursing
Dam centroid y
Dam convex hull roll mean 1s
Dam convex hull roll mean 2s
Dam-pup distance roll sum 1s
Dam-pup distance roll sum 2s
0.300
0.325
0.350
Passive nursing
Arm x
Arm y
Dam centroid y
Dam head centroid x
Dam head centroid y
0.03
0.06
0.09
Self-directed grooming
Side x
Dam centroid x
Dam head centroid x
Dam nose-back10 length
Dam nose-back10 length roll mean 0.1s
0.12
0.15
0.18
0.21
Eating
Arm probability
Side x
Dam head centroid x
Armpit movement roll mean 2s
Armpit movement roll sum 2s
0.12
0.16
0.20
SHAP score change from base rate
Drinking
Arm x
Side x
Dam head centroid x
Dam head centroid y
Back ellipse height
0.050
0.075
0.100
0.125
SHAP score change from base rate
Feature value
Low
Mean
High

B


Sum of SHAP scores by category
Nest attendance
Licking and grooming
Active nursing
Passive nursing
Self-directed grooming
Eating
Drinking
Pup coordinate
Pup area
Dam coordinate
Dam area
Dam distance
Dam movement
Dam-pup distance
-0.2
0.0
0.2
0.4
Sum of SHAP scores


A
Total duration
B
Percent time distributions
Nest attendance
Licking & grooming
Active nursing
Passive nursing
Self-directed grooming
Eating
Drinking
Time (s)
Number of videos
Postnatal day
Percent time