Development of a new 3D tracking system for multiple marmosets under free-moving conditions

NEW TRACKING SYSTEM FOR FREE-RUNNING CONDITIONS MARMOSETS

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Abstract

Accurate assessment of behavioral changes and social interactions in mammalian models is necessary to elucidate the pathogenesis of neurological disorders. The common marmoset (*Callithrix jacchus*) is a useful model in this regard. However, behavioral measurements, including assessments of sociality of free-moving group animals, have not been conducted in marmosets. Here, we developed a new behavioral analysis system for three-dimensional (3D) trajectories of independently free-moving multiple individuals by combining video tracking, 3D coordinates detected using light detection and ranging (Lidar), and facial recognition. Each marmoset was identified using deep learning facial recognition (accuracy ≥ 97%). Location preferences and distances between individuals were calculated using 3D trajectories, and grooming was detected using deep learning. This system will allow quantification of individual captive group animals, facilitating automatic measurement of social behavior. Furthermore, the behavioral changes observed might be directly extrapolated to humans and contribute to better understanding of the mechanisms underlying neurodegenerative disorders.

Introduction

Neurological disorders, such as neurodegenerative diseases and psychiatric disorders, are associated with decline in cognitive function and increases incidence of abnormal behaviors, which fundamentally affect social life[1, 2, 3, 4]. Mouse models, including genetically modified mice, have contributed to our understanding of brain structures, elucidating mechanisms of the onset and progression of neuronal disease, and behavioral changes caused by diseases. However, because of the anatomical and physiological differences between rodents and humans, mouse models do not fully recapitulate the pathogenesis of neuronal diseases. Therefore, there are limitations to using mouse models to address questions around human brain function[5, 6, 7]. In contrast, nonhuman primates do share anatomical and physiological features with humans, especially in specialized functions acquired through unique cerebral expansions, such as tool use, language, and self-awareness[7, 8, 9, 10]. As these functions are particularly affected in neurodegenerative diseases and psychiatric disorders, nonhuman primate models are crucial for understanding the development of neuronal and psychiatric disorders.

The common marmoset (*Callithrix jacchus*) is a small nonhuman primate with behavioral and social characteristics resembling those of humans. They are diurnal, form monogamous family units, engage in altruistic behaviors such as food sharing, and cooperate with all family members to raise their youngest offspring[9, 11, 12]. As marmoset body size and social units are small, maintaining complete social units for research in a laboratory is easier than that for other primates. Therefore,

52 marmosets are an ideal model for studying social behaviors in terms of maintaining families as social
53 units[9]. Recent advances in genetic modification techniques in nonhuman primates have enabled the
54 production of several genetically modified disease-model marmosets, such as those for
55 neurodegenerative diseases[13, 14, 15]. Using a marmoset model has advantages over rodents as model
56 animals and may help elucidate basic gene functions in the brain, clarify molecular mechanisms
57 underlying neurological disease onset using genetic modifications, and aid in implementing cognitive
58 neuroscience techniques in primate research[7, 16]. For these purposes, national brain research projects
59 using marmosets have been conducted in the US and Japan[17, 18].

60 Behavioral analyses are important for the validation of marmoset disease models. Various
61 behavioral task tests have been conducted for marmosets, including those using touch panels, tool
62 tasks, and evaluation of muscle motility[8, 19, 20, 21]. However, when a novel disease model is developed,
63 it is unclear when and how behavioral changes and social interactions will arise due to the disease.
64 Even if multiple types of exhaustive behavioral task tests are conducted, it is not always possible to
65 accurately capture behavioral changes through these tests. Further, it is often challenging to detect
66 behavioral changes, such as increased appetite, hypoactivity, aggression, irritability, and apathy, using
67 behavioral task tests. It is therefore important to clarify various behavioral changes, including changes
68 in social interactions, in animal disease models based on their behavior during free activity. In mice,
69 there are some systems for analyzing animal behavior during free-ranging activities under group-

70 housed conditions[22, 23, 24]. However, unlike mice, marmosets move quickly in three dimensions (3D),
71 thus mouse-specific systems cannot be directly applied to marmosets. Marmoset behavior tracking
72 measurement systems under free-moving activities have been previously reported, such as a sleep
73 study using an activity meter, head and eye tracking, and tracking of a single individual using a depth
74 sensor[25, 26, 27]. However, the activity meter and the head and eye tracking are limited because they
75 cannot track animal trajectories. The behavior tracking measurement systems using the Kinect system
76 depth sensor cannot analyze intra-familial marmosets under free-moving conditions because it does
77 not support simultaneous analysis of multiple animals and requires the use of a dedicated analysis
78 cage. Another method using infrared motion sensors has been reported[28]; however, it is limited to two-
79 dimensional motion and cannot detect the position and behavior of multiple marmosets under free
80 motion. Therefore, a novel system that can automatically and accurately detect and quantify the
81 individual behaviors of multiple marmosets under free moving condition to detect various behavioral
82 changes, including changes in social interactions is required.

83 Automatically detecting marmoset trajectories, various behaviors, and interactions
84 between individuals under free-moving conditions in multiple animals in a cage is especially
85 challenging, as there are many issues absent from other behavioral task tests or the quantification of
86 behaviors in single animals. For example, marmosets are often close to each other when grooming,
87 mating, or fighting[29]. This proximity makes it difficult to detect individual bodies, which complicates

individual tracking and behavior detection. Furthermore, movable objects, such as toys for environmental enrichment and food dishes, in an animal's home cage can also obstruct tracking. Although there are many challenges, analysis in the home-cage environment has the advantage of avoiding the stress caused by an unfamiliar experimental environment when observing family behavior. There have been previous attempts of pose estimation and tracking of multiple marmosets. However, successful long-term simultaneous tracking of multiple animals without labeling has not yet been achieved[30].

Here, we developed a 3D tracking technique using a combination of a light detection and ranging (Lidar) system and deep learning to simultaneously track a marmoset family of three in the same cage. To match the tracking trajectory with the individual animal information, facial recognition was performed to identify individuals and analyzed where each animal stayed in the cage, how long it stayed, and the distance between individuals. Furthermore, as an example of social behavior detection, we performed to automatically detect grooming behaviors using deep learning. The technology developed in this study will have applications in detecting important behavioral changes caused by a variety of diseases in small nonhuman model primates.

Results

Marmoset Facial recognition by deep learning

Five marmosets of the same generation (1-2 years old) were used to examine whether deep learning for face identification could be adapted to marmosets. These marmosets were kept in different cages in a conventional breeding colony and facial images were collected individually. To evaluate the trained models, 500 new facial images (100 for each animal) were obtained from the videos not used for training. The evaluation metrics for deep learning were as follows: specificity of 99.8%-100%, accuracy of 99.4%-99.8%, recall of 98.0%-100%, precision of 99%-100%, and an F-measure of 98.5%-99.5% (Table 1). The overall identification accuracy (total number of correct images/total number of images) of the five marmosets was 99.2%.

Next, face identification accuracy was examined in a family of three, which was raised in the set-up home cage. The trained model was evaluated using data that was not used for training. For the evaluation, 5,600 facial images (1,400 for each class), three classes including each marmoset, and an unknown class were used. The unknown class was used to exclude inappropriate images for identification, such as unclear images, images of the back of the head and faces in profile, and images not showing faces. The evaluation metrics for deep learning for individual identification were as follows: specificity of 98.7%-99.7%, accuracy of 98.6%-99.2%, recall of 96.1%-98.8%, precision of 98.4%-99.1%, and an F-measure of 97.3%-98.3% (Table 2). The overall percentage of correct

identifications was 98.0%.

Multiple animals' 3D tracking

To optimize the Lidar for laser measurement and video recording, a part of the cage made of metal mesh was replaced with acrylic panels, after which good measurement results were obtained (Fig. 1a). The laser beam distance needed to be sufficiently narrow and cover the entire cage, thus, the Lidars were placed 1000 mm from the front of the cage (Fig. 1b). All 3D tracking and behavioral tests were conducted using a single family consisting of three marmosets (male, female, and juvenile) in a cage.

The 3D coordinate information of marmosets was obtained using video tracking and Lidar (Fig. 2a-f, and Mov. S1). A raw image taken by the upper left video camera, capturing the entire cage and the three marmosets (Fig. 2a) and the data acquired by Lidar at the same time (Fig. 2b). The centroid of cluster by Lidar was indicated in white points, and each point is given a sequential tracking ID number during background processing. A new number was assigned each time the trajectory was broken. The 2D pixel location of the marmosets were then determined using an object detection algorithm in the video image (Fig. 2c). The same process was applied to the four cameras to determine the location of the marmosets for each camera. The 3D location of each marmoset was calculated using pre-calibrated camera positions and the pixel coordinates of the marmosets (Fig. 2d). The faces of the marmosets extracted by the object detection algorithm were identified using the trained VGG19

model, and individual IDs were assigned to each marmoset by facial recognition (Fig. 2e, f). These series of processed video data are shown in Mov. S1. In some cases, the marmosets could not be recognized by video tracking when they moved too fast or behind an object. In such cases, the trajectory was interpolated by the Lidar (Mov. S2, S3). Fig. 3 shows 5 min of tracking trajectories without face identifications (Fig. 3a, b) and with face recognition (Fig. 3c). When Lidar alone or Lidar and video tracking were performed, the tracking serial numbers changed frequently, and it was impossible to continuously track the individual (Fig. 3a, b). However, by applying face recognition to Lidar and video tracking, the trajectory of each animal was continuously detected, and marmoset IDs were assigned (Fig. 3c).

Trajectory analysis using the 3D tracking system

Using the results of 3D trajectories from this 3D tracking system, we analyzed where and how long each animal stayed in the cage during the one-hour period of the analyses (Fig. 4). This data indicated that the male (father) stayed on the lower floor of the cage for 80.2% of the one hour, while both the female (mother) and juvenile stayed on the lower floor for 0.4% of the one hour. On the other hand, they stayed on middle floor for half of the one hour and actively moved in a variety of areas in the cage except lower floor rest of the time (Fig. 4a-c, Table 3, Supplementary Fig. 1). Furthermore, the individual distances were analyzed using the location data (Fig. 4d). During this time, the distance

158 between the juvenile and female was less than 0.5 m 25.9% of the total time. However, a distance of
159 less than 0.5 m between juvenile-male and male-female was detected 1.0% and 2.2% of the time,
160 respectively. Conversely, the juvenile-female distance was separated by more than 1 m 2.0% of the
161 time, while the juvenile-male and male-female distance were separated by more than 1 m 20.0% and
162 11.0% of the time, respectively. From these results, it appeared that the juvenile preferred to be closer
163 to the female than to the male.

164 The same analyses were performed for long-term analysis on a different day during the
165 daytime, 7:00 am-7:00 pm. (Supplementary Fig. 2). After 6:00 pm, all marmosets moved to the bed
166 and clustered together and their faces were not observed, therefore, the data were excluded from the
167 analysis. The detailed analyses indicated that, among the family members, the juvenile stayed on the
168 lower floor of the cage the longest, unlike the results of the 1-hour analysis (Supplementary Fig. 3).
169 The time spent on the lower floor of the cage between 7:00 am and 6:00 pm for each individual was
170 male 8.6%, female 13.0%, and juvenile 23.5%. Throughout the day, the juvenile spent the most time
171 on the middle floor (27.7%), the male on the upper floor (29.7%), and the female on the upper floor
172 (29.9%). Furthermore, locations of each animal were analyzed hourly for 12 hours (Supplementary
173 Fig. 2, 3). The male also spent most of his time in the upper area in the morning (9:00 am; 54%), but
174 gradually changed his activity area to the middle area in the afternoon (1:00 pm; 51%) and the lower
175 area in the evening (4:00 pm; 45%). The female rarely stayed in the middle area but moved to the

upper section in the morning (9:00 am; 48%) and to the lower section in the afternoon (3:00 pm; 55%). The juvenile moved most evenly throughout the day among the three in cage. At night, all individuals moved to the upper bunk to move to their beds (Supplementary Fig. 2).

The distance between the marmoset pairs was also analyzed throughout the day. The results showed the male and female were closer than 0.5 m for 21.1% of the daytime. However, the male and the juvenile, and female and juvenile were closer than 0.5 m for 19.6% and 16.7%, respectively. Additionally, the closest and farthest pairs per hour switched frequently throughout the day (Supplementary Fig. 4).

Detection of behavior

In this system, behaviors could also be detected using video data from the two upper cameras in front of the cage (Supplemental Fig. 5a, b). Grooming behavior, one of the representative social behaviors of marmosets, excluding self-grooming behavior was detected. Training data on grooming behavior were obtained through observations of video data by skilled animal caretakers. The model was trained by annotated data of the grooming positions in each video frame.

The coordinates of the grooming location and individuals were detected by separately using the 3D tracking system, and determined which individual performed the grooming. Grooming behaviors were observed more often in the early morning (7:30 am-8:00 am), midday (12:30 pm-1:30

pm), and evening (3:30 pm- 4:00 pm, 5:00 pm), and all marmosets performed grooming behaviors equally (Fig. 5a). All marmoset groomed at fixed locations (Fig. 5b), namely the two upper areas of the cage. These two places were different from the sleeping area (Supplemental Fig. 2).

Accuracy Verification of the 3D tracking system

To determine the accuracy of the 3D tracking system, marmosets wore a color belly band to identify each animal, and individual tracking was conducted for 60 min. The video Mov. S4 shows a portion of this experiment and confirms that the system could track the marmosets accurately even when they crossed. Although the marmoset cage had a swinging hammock and balls as enrichment (Mov. S4, S5), these movements did not affect the tracking accuracy of the marmosets when using a combination of video tracking and Lidar. During this experiment, the marmoset IDs switched to different IDs nine times for more than 5 sec, but seven of the nine ID switching events were resolved within 15 sec. The maximum time length for accurate individual tracking was 19 min. Incorrect marmoset IDs of longer than 5 sec were exhibited 4.6% in the total 60 min.

The accuracy of the 3D trajectory data analysis in detecting the positions of animals in cages was confirmed by comparing it with visual observation data (Table 3, Supplemental Fig. 1). For example, in the visual observations of an hour, the juvenile was on the upper wooden bed 9.4% of the time, and on the middle floor 63.8%, on the lower wooden bed 25.1%, on the lower floor 0%, and in

the other areas 1.7% of the time. Similarly, the system-analyzed data showed that the upper wooden bed was 11.7%, the middle floor was 58.6%, the lower wooden bed was 24.1%, the lower floor was 0.4%, and the other areas was 5.2%. A strong significant correlation was found between the visual observation and system data ($R^2 = 0.959$, $P < 0.01$; Fig. 6a).

The accuracy of grooming behavior detection by the machine was compared with that of visual observation for one hour (Fig. 6b). Each 30-second video was considered as one unit. Twenty-four grooming units were visually detected, and 21 units were detected by the machine. Among these, 19 units were detected by visual observation and machine (recall: 79.1%). In the system, 19 of 21 units were confirmed as actual grooming behaviors (precision: 90.5%). The five missed units were difficult to detect because of the placement of animals behind an object: four had marmosets grooming behind the feeder, and one had grooming in the blind spot of the bed. Conversely, two units were detected by the machine but not by direct observation. During these events, the marmoset nuzzled its face into the other's tail or placed its hand on the other's shoulder. These actions seemed to be part of grooming behavior; however, observers did not judge grooming behaviors because they were not typical grooming actions.

Discussion

This study is the first report of simultaneous 3D tracking of multiple unmarked, freely moving nonhuman primates, using a novel approach that combines Lidar, video tracking, and facial

recognition through deep learning. Although these are all existing technologies, this is the first study that combines these technologies to achieve a 3D tracking system for small, high-speed objects. Since marmosets are social animals, maintaining them in a family unit is ideal for detecting natural behaviors during behavioral analysis. Our 3D tracking system made it possible to analyze the natural behavior of individual animals in a family group.

Owing to its detection speed and resolution, Lidar is suitable for application to fast-moving objects. Therefore, fast-moving marmosets are especially suited for tracking by Lidar. However, it falsely recognized objects that were similar in size to a marmoset, such as a marmoset tail or feeder boxes. Video tracking does not have this issue because it uses color image data to identify objects (Mov. S5). However, video tracking lost detection when marmoset motion was quicker than the camera's shutter speed or when the marmosets were behind obstacles (Mov. S2, S3). By compensating for the respective shortcomings of Lidar and video tracking and using each to complement the trajectories of the other, a tracking system for small, high-speed objects in three dimensions was realized.

Although either laser or video data can be used as the primary data for this system, in this study, we used video tracking as a base for detecting marmoset positions and then completed the missing information using Lidar, thereby compensating for the lost marmoset trajectory detections by video and false recognitions by Lidar.

In this study, a hammock and colored balls were placed in the family cage as the environmental enrichments. Although these enrichments were not firmly fixed to the cage, the hammock was not mis-detected by either Lidar or video because of different in size and shape from the marmoset, and it did not affect the tracking of the marmosets. Conversely, the balls were mis-detected by Lidar because of its similar size to marmosets, but not by video tracking (Mov. S5). Thus, although Lidar is useful for supplementing 3D information and creating trajectories, it is difficult to operate independently in a marmoset home cage with moving objects.

In addition, because both Lidar and video tracking can be processed at high speed without a time lag, no accumulation of data for later analysis occurs, which is favorable for long-term analysis. Indeed, the 3D trajectories and behaviors of three marmosets could be continuously analyzed in their home cage for over six months without data accumulation. The animals were healthy throughout the experiment, proving the safety of our system.

A similar 3D tracking system, the Kinect system, has been reported for marmosets[26] and similarly sized animals, such as rats[31] and pigeons[32], but its resolution is neither sufficient nor suitable for tracking fast-moving[22] or multiple animals. Another animal behavioral analysis system, DeepLabCut, is a powerful tool for estimating the posture of an animal using only a camera, but its accuracy depends on the shutter speed of the camera[30, 33]. Although the images in this study were captured at 30 Hz, the subjects were blurred because the marmosets were very active and moved

quickly. When used together with Lidar, accurate tracking was possible, even during jumps, without the need for a high-performance camera. OpenMonkeyStudio[34], an automated, marker-less pose estimation method for freely moving macaques using 62 machine vision cameras, has been also reported. OpenMonkeyStudio measures the natural behavior of macaques, aims to capture lifelong behavioral changes under free-ranging conditions, and can track multiple animals. However, this model is specific to macaques and, as previously mentioned, video tracking alone is not suitable for fast, 3D marmoset behaviors.

When marmosets are kept as a family, they repeat the behavior of huddling and then suddenly dispersing several times a day. This characteristic behavior makes it difficult to accurately track individuals using only either camera or Lidar. Therefore, we combined our system with facial recognition, using deep learning to detect and track each animal in a family group. Marmosets are usually identified individually using techniques including microchips, hair clippings, hair dyes, and necklaces[35]. Microchips and necklaces are unsuitable for MRI analysis, hair clippings and hair dyes require periodic maintenance, and necklaces risk tightening around the neck as they grow or getting caught. In part of this study, the marmosets were identified as individuals by wearing a belly band, but the female did not like the band and was constantly fidgeting with it. Thus, artificial markers risk causing unexpected behavioral changes. Therefore, the goal of this project was to achieve marker-less identification of individuals. Since the most distinctive feature of the marmoset's body is its face, the

face was used as a marker for individual identification.

The marmoset family used in this study had no blood relationships with other family members. When pairing marmosets, we selected unrelated individuals to avoid inbreeding and maintain genetic background diversity in the colony. In the marmoset family used in this study, the male (foster father) was vasectomized, and the juvenile (son) was obtained by embryonic transfer of the embryo from the embryo donor pair of marmosets into the female's (foster mother) uterus[15, 36]. Regarding facial identification, the most accurate results within the family were those for the juvenile (Table 2); although the juvenile was beyond sexual maturity, young animals have easily identifiable distinctive facial features. In addition, when using five animals of the same age (1–2 years old), the animals could be identified; therefore, highly accurate classification is possible by facial recognition even when the animals are of similar age (Table 1). Incorporating facial recognition results into the trajectory enabled long-term tracking, even for multiple animals in the same cage (Fig. 3).

We examined the accuracy of marmoset tracking using this system by color labeling the marmosets and comparing this to visual tracking (Mov. S4, Table 3). This experiment confirmed that it is possible to track animals using facial recognition, with 95% accuracy, without labeling marmosets or relying on color information.

In addition, using the 3D coordinates data, this system could calculate where each animal stayed in the cage, how long it stayed (Fig. 4a-c, Table 3, Supplemental Fig. 2, 3), and the distance

303 between individuals (Fig. 4d, Supplemental Fig. 4), which suggests interactions between individuals.
304 Although switching of the marmoset IDs were observed several times during the hour-long experiment,
305 a comparison of the visual observations and system analysis results for where each marmoset existed
306 in which 3D coordinates in the cage, and how long each marmoset spent in that area was highly
307 correlated (R^2 = 0.959, Fig. 6a, Supplemental Fig. 1). Indicating that the slight ID switching did not
308 affect the experimental results, and our tracking system was sufficiently accurate for automatically
309 analyzing marmosets' 3D coordinates in the cage.

310 Marmosets often change their preferred position and the individuals they spend time with
311 during the day. For example, the male marmoset preferred the upper area in the morning, but in the
312 afternoon, it preferred the lower bed. This result was not apparent in the 1-hour analysis compared
313 with observations. Furthermore, one-hour and 12-hour analyses of position in the cage and distance
314 between marmoset individuals showed different results. For example, a one-hour analysis showed that
315 the male preferred the cage's lower floor, and remained there 80% of the analysis time (Table 3).
316 However, a 12-hour analysis indicated that the juvenile spent the longest time (23.5%) on the lower
317 floor (Supplemental Fig. 2, 3). The analyses of the distance between individuals showed that female
318 and juvenile were within 0.5 m for 25.9% of the one-hour analysis, but the 12-hour analysis indicated
319 that the male-female remained closer than the female-juvenile or male-juvenile. These results suggest
320 that the results of a human observation for a limited time and a machine observation throughout the

day may differ. Indeed, when we directly observed this family, the male and the female were usually far apart, but a 12-hour analysis suggests that the male and female spent time close together. Therefore, it should also be considered that people's presence alters marmoset behavior. To correctly understand marmoset behavior, it is necessary for the behavior analysis system to constantly analyze a marmoset's life with the same evaluation criteria. Our 3D tracking system can analyze animal positions and distances between individuals all the time.

In addition, some marmoset facilities, to provide more space or different environments for marmosets, sometimes connect adjacent marmosets' cages[37]. In this study, an extra cage was connected to the 3D tracking cage. When a marmoset went off-screen due to movement to the connected cage, the tracking of the marmoset was temporarily interrupted. However, other marmosets continued to be tracked, and none were tracked instead of the missing marmoset (Mov. S6). Tracking was then resumed when the marmoset returned to the cage.

Grooming, a typical social behavior, was detected through deep learning using video analysis. Comparing detection of the grooming behavior using visual observation by humans and the system showed that grooming behaviors were detected with high precision (90.5%) and recall (79.1%; Fig. 6b). In some cases, the system identified behaviors just before or after the grooming as grooming behaviors. The deep learning system may have detected certain behaviors that might be unique to periods before and after grooming. However, in other cases, grooming behavior could not be detected

339 by the deep learning system because the groomers were in the camera's blind spot. Therefore, the
340 training dataset must be further updated to detect behavior behind obstacles. Similar methods may
341 also be used in the future to detect a variety of other behaviors. Combining the 3D tracking with the
342 behavior detection by deep learning, this system can reveal when, where, and what behavior of each
343 marmoset by accurate quantification, and the social relationships among the animals (Fig.5 a, b). The
344 12-hour grooming behavior analysis in this study indicated that grooming behavior was found to occur
345 at specific places: upper beds and floor, and at specific times: early morning, midday, and evening.
346 This finding was only obtained using automatic behavior analysis. While there are existing methods
347 for automatically detecting the behavior of a single animal[38], our system is able to detect the behavior
348 of multiple animals in captivity and identify the individuals and their locations for the first time.

349 In conclusion, the combination of Lidar, video tracking, and facial recognition enabled
350 successful simultaneous tracking of each marmoset in a family without artificial labeling. Using data
351 from the 3D tracking system we developed, we can measure 3D coordinates, movement trajectories,
352 the quantity of activity, position in the cage, when, and for how long each animal stayed, distances
353 between individuals, and behavior detection. These measurements will allow automatic analysis of the
354 activities and behaviors of marmosets under free-moving conditions throughout their lifetime.
355 Importantly, these data will be useful to identify early parameters for various diseases and to evaluate
356 the pathology of disease-model marmosets. In animal models of autism spectrum disorders and

Alzheimer's disease, abnormalities in social interactions have been reported, and differences in distance and position between mice have been observed[39, 40, 41]. Therefore, in genetically modified marmoset models of Alzheimer's disease and other diseases that develop over long periods, the distance between marmosets and their location of preference may serve as early markers for the onset of the disease[15]. Moreover, Lidar can track the location of animals' day and night; therefore, their nocturnal behavior can be observed to obtain primary data. Sleep disorders have recently been suggested as a predictor of Alzheimer's disease and Parkinson's disease [42, 43]. This system would be able to accurately quantify the behavior of marmosets throughout their lives to discover behavioral changes before and after the onset of diseases, even at an extremely early stage. Recently Calapai et al. reported a new touch screen based cognitive auditory analysis system that allows marmosets to perform tasks in a free moving environment in the paired home cage[44]. By incorporating such a freely operable cognitive function task device into our system, it is possible to provide a behavior analysis system that comprehensively and continuously analyzes the marmoset's natural behavior, activity, and cognitive functions without stress. These behavioral changes might be directly extrapolated to humans and contribute to a better understanding of the mechanisms of neurodegenerative and psychiatric disorders.

Materials and Methods

Hardware Setup

The home cage was made of acrylic panels on the front and back, and all food dishes, water jugs, and bedding inside the cage were fixed to the wire mesh on the side to prevent them from moving. This cage was designed to conduct behavioral analysis in a marmoset's home cage (Fig. 1a). Four poles were set up in front of the cage at 1000 mm, and four Lidars (VLP-16, Velodyne Lidar, San Jose, CA, USA) and four cameras (C920, Logitech, Lausanne, Switzerland) were fixed to the poles (Fig. 1b). The Lidar was controlled by motion measurement software, the path traced, and velocity of the marmosets recorded (Hitachi, Ltd, Tokyo, Japan.). The laser beam of the Lidar was irradiated radially and adjusted to cover the entire inside of the cage. The recording parameters of the cameras were 1080p and 60 Hz, and all cameras were adjusted to show the entire cage (Supplemental Fig. 4). The positions of the cage, Lidar, and cameras were calibrated when the cage was moved, and the interiors were changed. Lidar calibration was used to acquire background information, including the cage interior without marmosets. Calibration of the camera was performed by detecting alco-markers at the four corners of the cage.

Ethical approval

This study was conducted at the animal facility of the Central Institute for Experimental Animals (CIEA), Kawasaki, Japan. The animal experimental protocol was reviewed by the Institutional Animal

Care and Use Committee and approved (approval no. 17051) according to the Regulations for Animal Experiments in CIEA, based on the Guidelines for the Proper Conduct of Animal Experiments by the Science Council of Japan (2006).

Animal facilities

The marmosets were kept in a family cage with dimensions of 820 × 610 × 1578 mm. Although there was only one marmoset family in the room, they were able to vocally communicate with the marmosets in other rooms. All cages were equipped with a bunk, and the animal rooms were adjusted to 26-28°C, 40-60% humidity with a 12:12 h light/dark cycle. The animals were negative for *Salmonella* spp., *Shigella* spp., and *Yersinia pseudotuberculosis* on annual fecal examination. The animals in the subject were inspected nine months ago.

Animals

Each marmoset family was housed in a home cage. The observed family consisted of a male (I5072M, ten years old), female (I5894F, six years old), and a juvenile (I940M, one year old). No artificial or non-artificial physical features for individual identification were used, such as hair dye, necklaces, piercings, or other visible signs, except during 3D tracking of marmoset-attached color marker experiments. In conventional animal care, a microchip is implanted subcutaneously for individual

identification. The juvenile was born by embryo transfer to the mother's uterus[15], and there was no blood relationship within the third degree of the family.

Five healthy marmosets approximately 1-2 years old, one male and four female, were used in the preliminary study to develop deep learning facial recognition (I774 one year old, I6695 one year old, I6708 one year old, I6677 one year old, and I893 two years old). There were no artificial or visible physical characteristics, such as hair dye, necklaces, or piercings, and each marmoset was not related to any other marmoset within the third degree of kinship.

Facial recognition

The cage (W820 × D610 × H1578 mm) was divided into four compartments to isolate each marmoset for obtaining training images by video camera. Each animal was housed in a separate cage, and videos were recorded from the cage front for 3-6 hours. The front door of the cage was then replaced with clear acrylic panels. From these video frames, the face images were cropped only in the face area for the training images, and these data were augmented by rotation. Using 1400 facial images in each class for training, frontal images of the face were classified into four or five classes using the VGG19 model network. In the preliminary test, the five classes comprised five marmosets. For the family group, the four classes included those members and an unknown class to exclude images inappropriate for classification, and 1000 images were used for training.

Model training was performed by fine-tuning a pre-trained VGG19 model (learning rate 0.001, fixed number of layers 12, BATCH_SIZE 32)[45] (Supplementary Data S2). VGG19 is a network model proposed by the Visual Geometry Group at the University of Oxford in 2014 that obtained accurate classification performance on the ImageNet dataset. The pre-trained models used in this study were trained using the ImageNet dataset.

3D tracking

Lidar and video tracking performed 3D tracking. A Lidar measures the distance to an object by irradiating a laser and analyzing the reflected laser. Marmoset detection using Lidar involves the following steps: background information removal, clustering, and selection of marmoset clusters[46]. Background removal was performed during calibration. The observed points were moving points belonging to objects whose spatial positions changed and static points from the background environment. Marmosets were then selected from among all the moving clusters. The Euclidean cluster extraction algorithm was used for clustering, with a minimum cluster size of S1 and maximum cluster size of S2 (S1: 100 mm, S2: 500 mm)[47]. The centroid of the cluster is regarded as the position of the marmoset for tracking. Three-dimensional video tracking was performed using four time-synchronized video cameras, 3D video tracking was performed. The positions of the whole body and head of the marmoset were detected in each video frame using Yolo v3, an object detection algorithm,

and the 3D position was calculated from each camera information[48]. The Yolo model was trained using 4611 images in which the whole body and head area were annotated (learning rate: 0.0003, momentum: 0.9, batch: 64). For video tracking, 3D coordinates were used as primary information and supplemented with Lidar coordinates when the primary information was missing. The program for linkage between video tracking and Lidar for individual information was produced by Hitachi, Ltd. When the marmoset coordinates were detected outside the cage, they were considered missing values.

Detecting grooming behavior

Yolo v3, an open-source deep learning software, was used to detect grooming behavior. As training images, 252 annotated image files in which grooming marmosets were surrounded by a minimum rectangle were prepared, and the model was trained using the dataset. The trained model analyzed the top two camera view images and detected grooming behavior in each video frame. To improve the accuracy by removing noise, a Gaussian weighted moving average of 50 sec window and 5 sec slide was applied to the frequency of grooming occurrence each time to create the probability distribution of grooming occurrence. The same process was performed for the left and right cameras and the calculated probability distributions were averaged. When the average value exceeded a certain threshold, grooming was considered to have occurred (Supplementary Data S2). To test the accuracy of this detection system, skilled breeding staff visually detected marmoset grooming in one hour of video that was not used for training data. This visual result was used as the true label, and the accuracy

and repeatability of grooming detection were calculated in 30 sec increments of time. Because grooming was analyzed with two cameras, it was possible to link location and time. Grooming location were calculated in the same way as for video tracking. The grooming time was based on the video file.

Statistical analysis

Pearson's correlation coefficient was calculated using GraphPad Prism version 9 (GraphPad Software, La Jolla, CA, USA).

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Author contributions

T.Y. and E.S. conceived and designed the study. T.Y., N.G., J.K., and T.F. performed the setup of the measurement system. R.K., R.H., M.K., Y.H., and E.Y. performed the annotation of the training data. T.Y., Y.S., R.H., J.K., and T.F. processed data, trained the models, and analyzed results. T.Y. and R.K. performed the experiment. T.Y., W.K., K.S., N.G., T.I., and E.S. discussed and interpreted the results. T.Y., T.I., and E.S. wrote the manuscript. E.S. supervised the project. All authors commented on the manuscript.

Competing interests

NG is an employee of Hitachi, Ltd. JK is an employee of Hitachi Solutions Technology, Ltd. TF is an employee of Totec Amenity Limited. The remaining authors declare no competing interests.

Resources Availability

Information and requests for the program for linkage between video tracking and Lidar should be directed to and will be fulfilled by Norio Goda (norio.goda.ws@hitachi.com). For other resources and information should be addressed to Erika Sasaki (esasaki@cieea.or.jp).

Data and code availability

The readily tested code and data has been presented in the Supplemental information (Supplementary Data S1, S2).

Figure 1. Installation of hardware for the behavior analysis system

(a) A home cage with acrylic panels on the front and back and metal mesh on the sides. (b) Schematic diagram of light and ranging (Lidar) systems and cameras installed in front of the cage. Lidars and cameras were installed 1000 mm in front of the cage.

Figure 2. 3D tracking of marmosets using Lidar and video tracking

(a) Raw video frame. (b) Detected 3D coordinates of marmosets by Lidar. White point: centroid of marmosets; white box: virtual space of the cage. (c) Detected location of marmosets in video frame

by Yolo. Rectangle: detected marmosets. (d) Calculated 3D coordinates of marmosets by video tracking, colored point: coordinate of marmoset. (e, f) Individual information added to c and d using face identification, green: I5072M, red: I5894F, yellow: I940M.

Figure 3. 3D position trajectory of a marmoset in five minutes

(a) Marmoset trajectories analyzed by Lidar. White points indicate centroid of marmosets. (b) Lidar and video tracking. Different colors were assigned to each tracking serial number that numbered the background. (c) Lidar and video trajectory tracking combined with face recognition. Green: male; red: female; yellow: juvenile.

Figure 4. Place preference of marmosets for 1 hour

Color map of the time spent by marmosets in the cage for one hour (a-c). Locations where marmosets spent more than 1% of one hour are shown in red, and others are shown as color bars. (a) Male (I5072M), (b) female (I5894F), (c) juvenile (I940M). (d) Histogram of the distance between each family member. Blue bars indicate the individual distance between the juvenile (I940M) and the male (I5072M), orange bars indicate the individual distance between the juvenile and the female (I5894F), and green bars indicates the individual distance between the male and the female.

Figure 5. Detection of grooming behavior for a day

(a) Number of grooming behaviors detected per day. Bars show the number of frames detected each 5 min. (b) The 3D coordinates where the grooming behavior of each individual was detected are shown. Red indicates juvenile (I940M), green indicates male (I5072M), and blue indicates female (I5894F).

Figure 6. Comparison of human visual observations and the system

(a) Each cell represented 30 s. Blue cells: Visual observation detected grooming at least once in 30 s. Red cells: The system detected grooming by the intensity exceeding a threshold value. (b) The time the marmosets stayed in a particular location was compared visually and by the system. The marmosets stayed in four locations: upper bunk, middle floor, lower bunk, and lower floor. Dotted lines indicate regression lines.

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Fig. 1

a

b

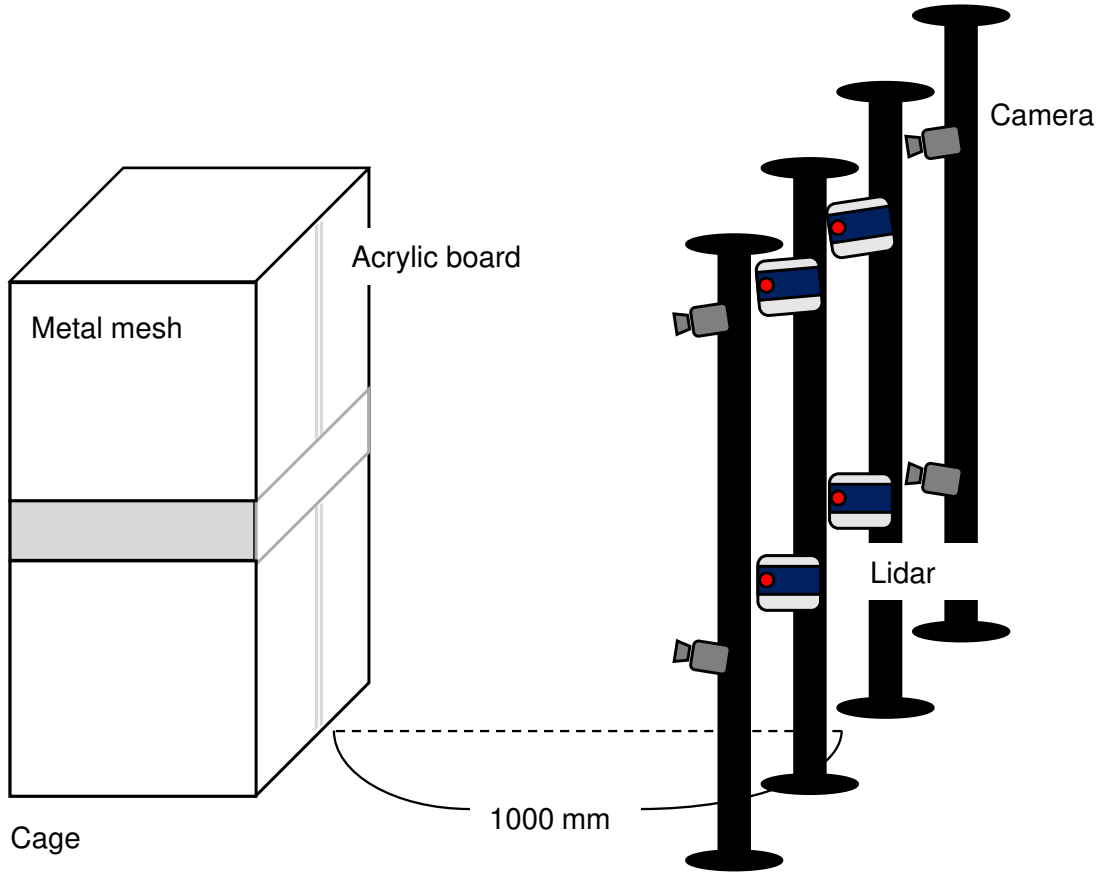


Fig. 2

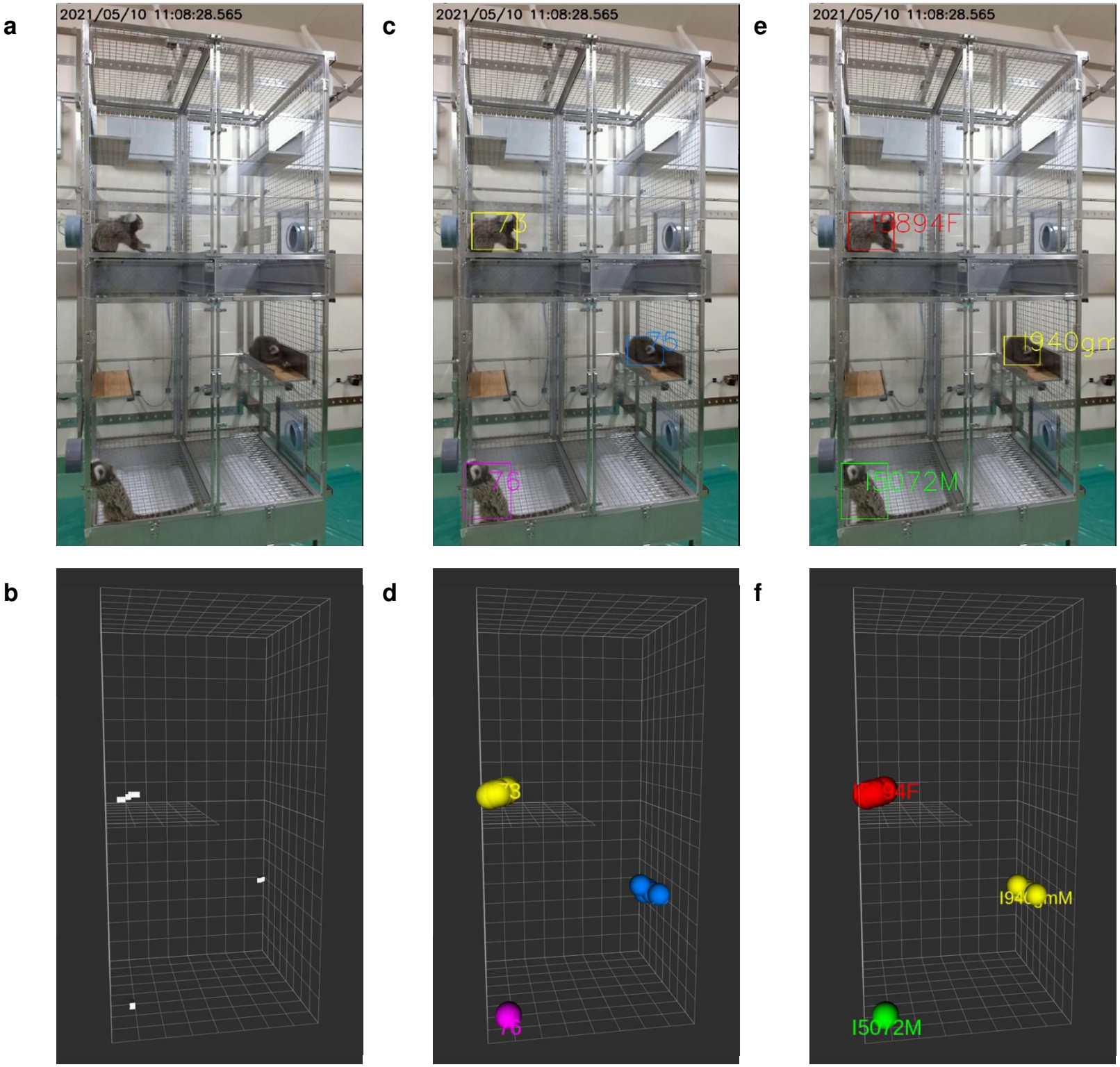

a
2021/05/10 11:08:28.565
c
2021/05/10 11:08:28.565
e
2021/05/10 11:08:28.565
b
d
f

Fig. 3

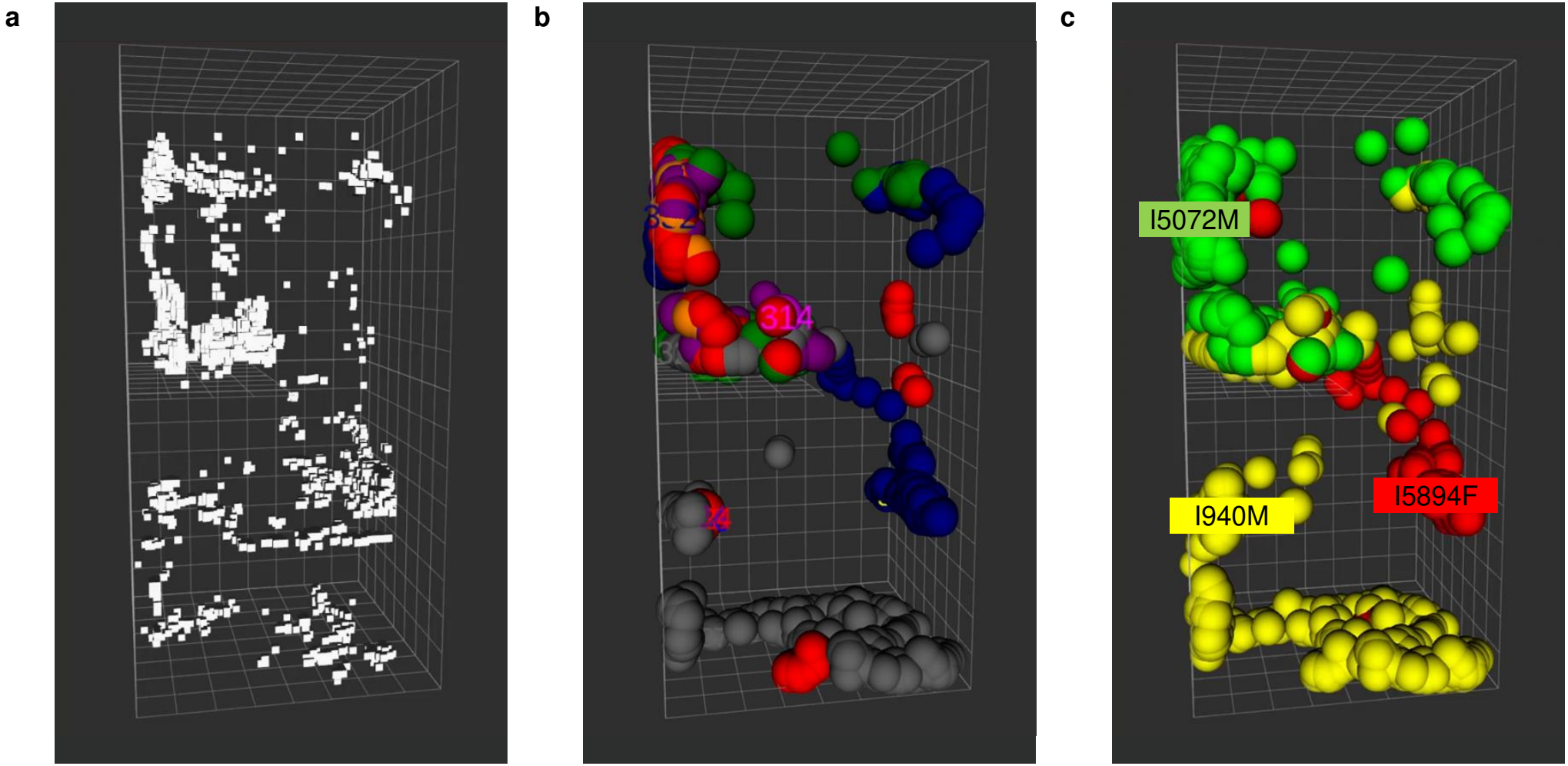


Fig. 4

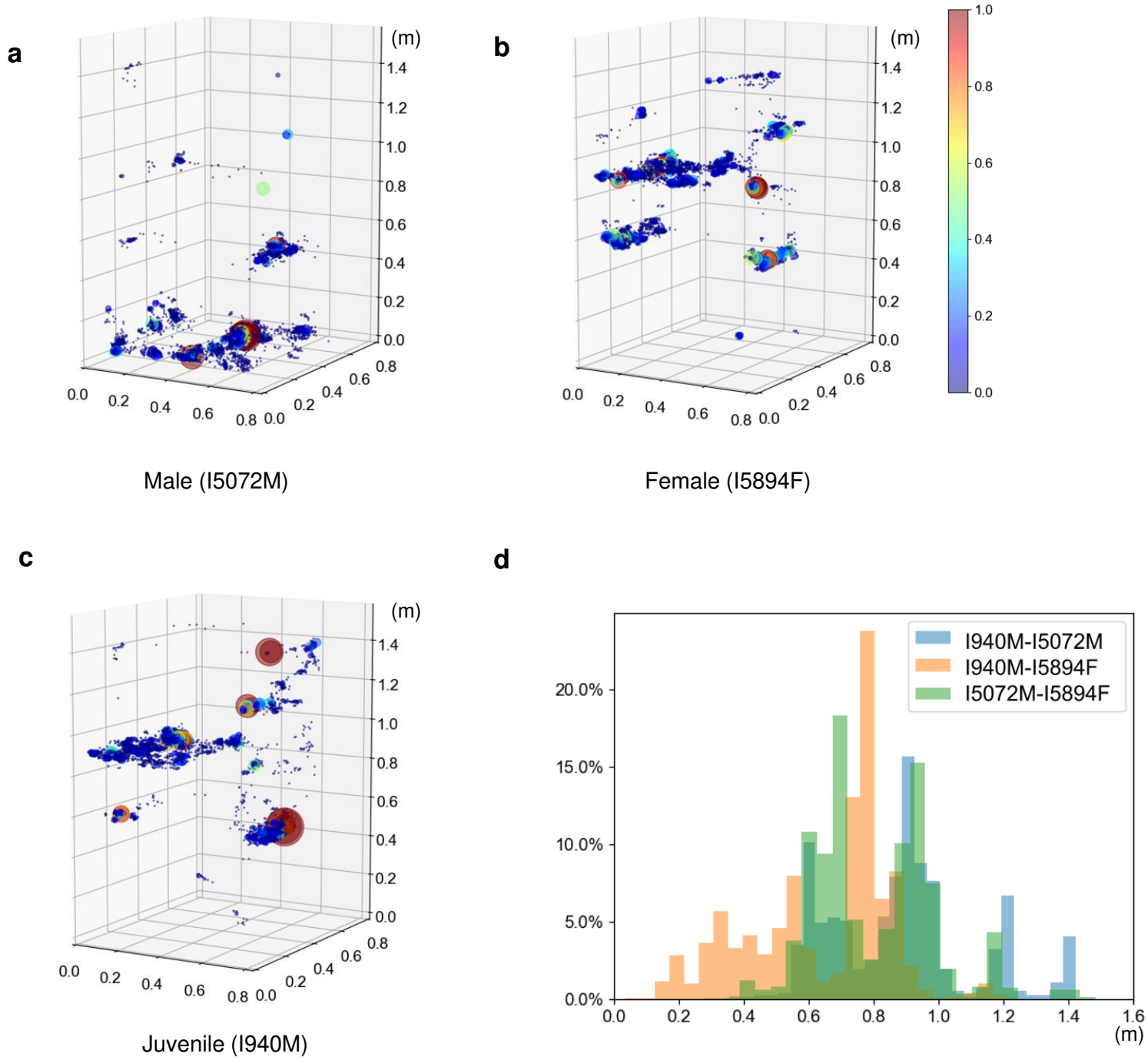
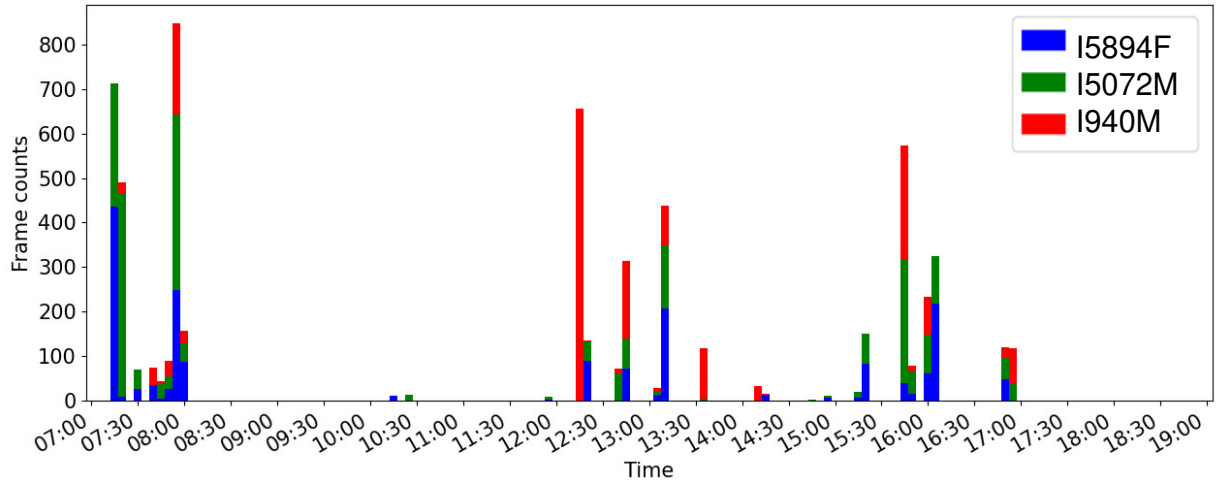

a
(m)
Male (I5072M)
b
(m)
Female (I5894F)
c
(m)
Juvenile (I940M)
d
I940M-I5072M
I940M-I5894F
I5072M-I5894F
(m)

Fig. 5

a


I5894F
I5072M
I940M
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100
200
300
400
500
600
700
800
07:00
07:30
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08:30
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13:00
13:30
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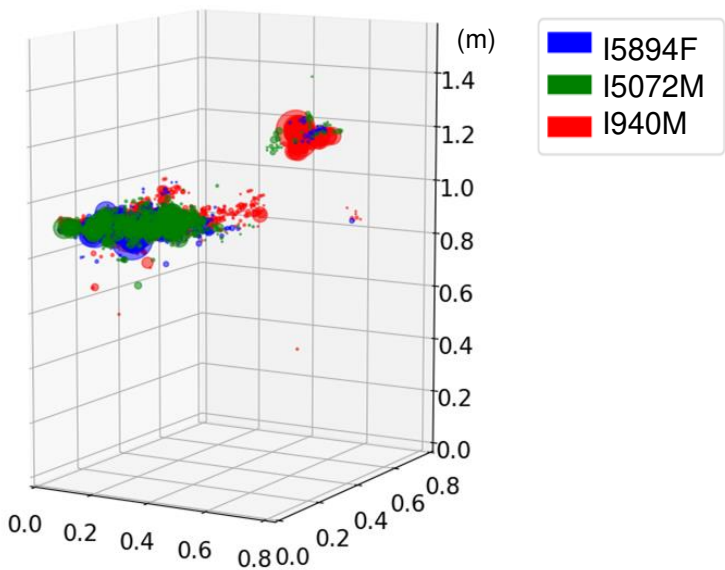
17:00
17:30
18:00
18:30
19:00
Time

b


(m)
I5894F
I5072M
I940M
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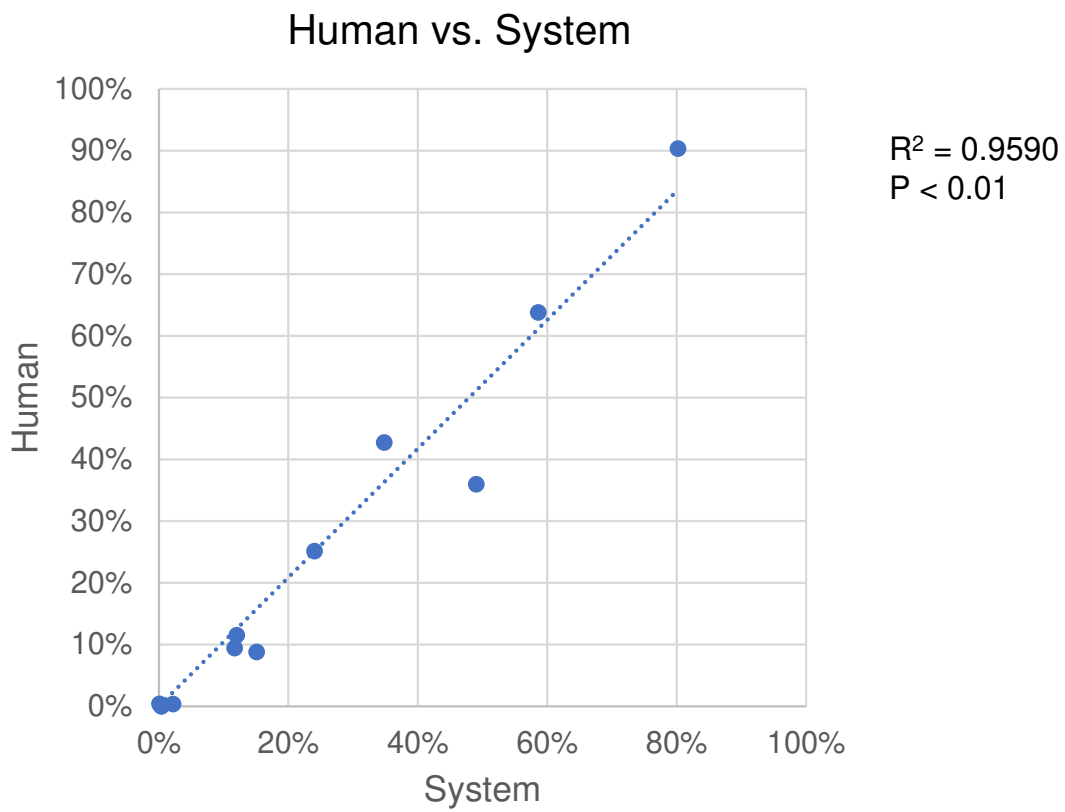
0.4
0.6
0.8
1.0
1.2
1.4

Fig. 6

a


Human vs. System
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Human
0%
20%
40%
60%
80%
100%
System
R² = 0.9590
P < 0.01

b

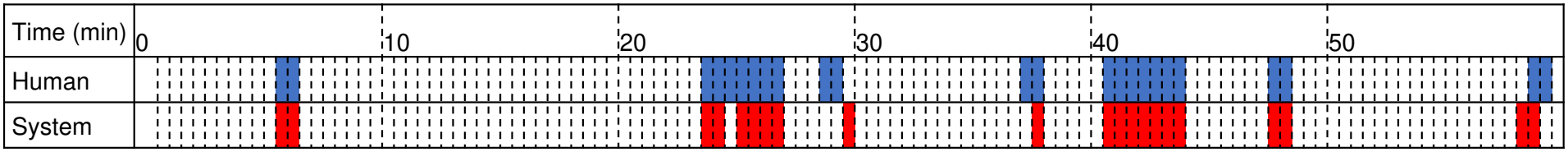

Time (min)
0
10
20
30
40
50
Human
System

Table 1. Classification accuracy of face identification in five marmosets aged 1 to 2 years.

	Animal ID				
	I774	I6695	I6708	I6677	I893
Specificity	100.0%	99.8%	99.8%	99.8%	99.8%
Accuracy	99.8%	99.6%	99.4%	99.8%	99.8%
Recall	99.0%	99.0%	98.0%	100.0%	100.0%
Precision	100.0%	99.0%	99.0%	99.0%	99.0%
F-Measure	99.5%	99.0%	98.5%	99.5%	99.5%

Table 2. Classification accuracy of face identification in a marmoset family.

	Male (I5072M)	Female (I5894F)	Juvenile (I940M)	Unknown
Specificity	98.7%	99.5%	99.7%	99.5%
Accuracy	98.7%	98.6%	99.2%	99.5%
Recall	98.8%	96.1%	97.6%	99.6%
Precision	96.1%	98.4%	99.1%	98.5%
F-Measure	97.4%	97.3%	98.3%	99.0%

Table 3. Comparison of visual observations and the system analysis of time spent in specific locations in the cage for an hour

	Male (I5072M)		Female (I5894F)		Juvenile (I940M)	
	Human	System	Human	System	Human	System
Upper bed	0.4%	0.1%	11.5%	12.1%	9.4%	11.7%
Middle floor	0.3%	2.2%	35.9%	49.1%	63.8%	58.6%
Lower bed	8.8%	15.1%	42.7%	34.8%	25.1%	24.1%
Lower floor	90.3%	80.2%	0.0%	0.4%	0.0%	0.4%
Other	0.2%	2.4%	9.9%	3.6%	1.7%	5.2%