Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) in press
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Research Article

https://doi.org/10.1631/jzus.B2400294

Establishment of a standardized daily behavior collection and analysis system for brain disease models of rhesus and cynomolgus monkeys and its application in autism spectrum disorder

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Abstract: Complex brain diseases seriously endanger human health, and early diagnostic biomarkers and effective treatments are currently lacking. Due to ethical constraints on human research, establishing monkey models is crucial to address these issues. With the rapid development of technology, transgenic monkey models of a range of brain diseases, especially autism spectrum disorder (ASD), have been successfully established. However, to establish practical and effective brain disease models and subsequently apply them to disease mechanism and treatment studies, there is still a lack of a standard tool, i.e., a system for collecting and analyzing the daily behaviors of brain disease model monkeys. Therefore, with the goal of undertaking a comprehensive and quantitative study of behavioral phenotypes, we established a standard daily behavior collection and analysis system, including behavioral data collection protocols and a monkey daily behavior ethogram (MDBE) for rhesus and cynomolgus monkeys, which are commonly used in model construction. Then, we used ASD as an application example, referring to the DSM-5-TR, which is widely used in clinical diseases and models, to obtain its core clinical symptoms. We then established a sub-ethogram (ASD monkey core behavior ethogram, MCBE-ASD) for quantitative assessment of the core clinical symptoms of an ASD monkey model based on MDBE. Subsequently, we demonstrated the high reproducibility of the system through

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Received June 6, 2024; Revision accepted Sept. 25, 2024;
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

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experimental data and compared the differences in behavioral patterns among rhesus monkey individuals.

Key words: Monkey daily behavior ethogram; Monkey behavior collection protocol; Rhesus monkey; Cynomolgus monkey; Autism spectrum disorder (ASD)

1 Introduction

In recent years, the incidence of various brain diseases has been increasing annually. This trend has placed a heavy burden on patients, their families, and society, posing a serious threat to human health (Feigin et al., 2021). In the case of autism spectrum disorder (ASD), for example, the US Centers for Disease Control and Prevention (CDC) reports that 1 in 36 children have ASD, a 4.14-fold increase since 2000 (Maenner et al., 2023). Data show that 60%–70% of individuals with ASD are unable to complete normal education, maintain a job, or live independently. The etiology of most brain diseases is highly complex, and the pathogenesis remains unclear (Lintas and Persico, 2009; Chaste and Leboyer, 2012; Ghosh et al., 2013; Ronemus et al., 2014), leading to a lack of effective diagnostic biomarkers and therapeutic strategies. Given the ethical constraints on human research, it is essential to develop animal models that can effectively replicate conditions in human patients in order to find solutions.

Non-human primates have a clear advantage over other experimental animals in mimicking human brain disease phenotypes due to their high similarity to humans in terms of genes, brain structure and function, and behavioral performance. Rhesus and cynomolgus monkeys—the non-human primates most frequently used in experimental research—share 93.5% genetic similarity with humans (Gibbs et al., 2007) and exhibit advanced emotional responses and complex behaviors similar to those of humans, including depression, anxiety, and restricted interests. Therefore, they are ideal animals for modeling human brain diseases (Kalin and Shelton, 2003; Chan et al., 2015; Qiao et al., 2023).

In recent years, with the rapid development of monkey gene editing technology, a series of transgenic monkey models of brain diseases have been successfully established. In particular, remarkable progress has been made in the establishment of ASD monkey models, as the ASD pathogenic genes have been identified, and their gene penetration and expressivity are high. For example, in 2016, Zilong Qiu's group successfully established the first lentivirus-transfected *MECP2* ASD cynomolgus monkey model (Liu et al., 2016). In 2017, Weizhi Ji's group successfully modeled the knockout of the *MECP2* gene in cynomolgus monkeys using TALEN technology, leading them to exhibit core symptoms of ASD, such as stereotyped behaviors and impaired social interactions (Chen et al., 2017). In 2019, Guoping Feng's team (Zhou et al., 2019) and Xiaojiang Li's team (Tu et al., 2019) each edited the *SHANK3* gene using CRISPR-Cas9 technology, both succeeding in inducing the classic ASD phenotype in cynomolgus monkeys. In 2021, our team knocked out the *MECP2* gene in the hippocampus of young rhesus monkeys and successfully induced the first in situ gene-edited ASD model (Wu et al., 2021). In addition to the ASD model, our team also knocked out both PINK1 and DJ-1 genes through in situ gene editing technology to establish the first adult rhesus monkey Parkinson’s disease (PD) model with typical clinical symptoms and pathological hallmarks (Li H et al., 2021). The establishment of these new models provides new platforms and opportunities for the research and treatment of brain diseases.

The biggest breakthrough in these models, when carefully analyzed, is the establishment of novel and effective gene editing techniques for monkeys. The transgenic models established with these novel techniques have, for the first time, opened the door to applying knowledge of the genetics of brain disease to studying its underlying mechanisms and potential treatments. However, to truly establish effective and practical brain disease models and apply them to disease mechanism and treatment research, another important technological breakthrough is needed—a standard, comprehensive system for collecting and analyzing the daily behaviors of monkeys modeling brain diseases. For example, when core clinical symptoms (behavior) were measured in the above five ASD models to determine whether the modeling was successful, different research teams used sig-

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nificantly different behavioral collection protocols and data analysis methods, which makes direct behavioral phenotype comparison between the models very difficult. This is also very unfavorable for comparing and improving future models, using models to study disease mechanisms, and evaluating the efficacy of drugs or intervention measures.

To achieve a direct comparison between different models and lay a good foundation for future behavioral phenotyping studies of various monkey models of brain diseases (not only ASD), based on the current breeding and rearing of gene-edited model monkeys in the cages of qualified facilities, this study established a standardized daily behavior data collection protocol and analysis system (ethogram) mainly for behavioral phenotyping studies of rhesus and cynomolgus monkey models of brain diseases. The logical basis for this is that if everyone uses the same tool (ruler) when evaluating model behavioral phenotype, the comparability of results is naturally guaranteed. The ethogram (a catalog or list of the observed behaviors of a particular species) of this system has the following two main goals: 1) to refer to the authoritative international clinical diagnostic manual for brain diseases in order to quantitatively evaluate the core clinical symptoms of brain disease models to determine whether the modeling is successful and 2) to break through the focus on core clinical symptoms and conduct as comprehensive a study as possible on the various daily behavioral phenotypes of model monkeys, as changes in a gene can have extensive and profound effects on daily behaviors, possibly far beyond the scope of clinical symptoms. This enlargement of scope can provide geneticists with a powerful tool to study the important biological issue of the gene–phenotype relationship and can also provide a broader and deeper behavioral basis for research on the mechanisms and treatment of brain diseases. Compared with the core clinical symptoms, the general clinical phenotypes of various brain diseases are rich and complex. To comprehensively evaluate them, the establishment of an ethogram with as comprehensive a behavioral phenotype as possible is required.

To achieve these goals, we developed a comprehensive ethogram that covers all the daily behaviors of rhesus and cynomolgus monkeys gathered from the literature. Then, we referred to the core clinical symptoms (behaviors) of various brain diseases, extracted and combined the corresponding behaviors from the comprehensive ethogram, and created a sub-ethogram that can be used to evaluate the core clinical symptoms of a specific brain disease (such as ASD) model to determine whether the modeling is successful. By recording and analyzing the frequency and time of various behaviors in the ethogram and sub-ethogram, a quantitative evaluation of the daily behavioral phenotypes of the model monkeys could be achieved. The system is applicable to a range of ages, including adolescent, adult, and elderly monkeys, but does not include infants or maternal behavior. Applicable species include rhesus and cynomolgus monkeys, both male and female.

The following is a brief review of the differences in the behavioral phenotyping systems used in the above five ASD gene editing models and their consequences, and then a solution is proposed, which is the principle for establishing the daily behavior collection and analysis system presented in this article. First, there were large differences in the specific timing and total duration of behavioral collection protocols among the five studies. In terms of the duration of behavioral collection, Qiu's (Liu et al., 2016) and Feng's (Zhou et al., 2019) teams did not give clear descriptions of the specific recording time points. Ji's team (Chen et al., 2017) chose to record at 9:00–10:00 in the morning, Li's team (Tu et al., 2019) recorded for 30 minutes at 9:00–11:30 in the morning and 14:00–16:30 in the afternoon, and our team (Wu et al., 2021) recorded for 30 min at 9:30–11:30 in the morning and 14:00–16:00 in the afternoon. In terms of the length of time for behavioral collection, Qiu's team (Liu et al., 2016) recorded the target monkeys' behavior for one hour every day for five consecutive days; Ji's team (Chen et al., 2017) recorded each monkey five times without specifying the duration; our team (Wu et al., 2021) and Li's team (Tu et al., 2019) both recorded continuously for 30 minutes each in the morning and afternoon. Our team (Wu et al., 2021) collected data for seven consecutive days, while Li's team (Tu et al., 2019) recorded for six consecutive days. It is well-established that monkey behaviors vary over time, showing various behavioral patterns at different hours of the day (Li Y et al., 2019). Different teams recording behaviors at different time points reduce the comparability of the data. In addition, if the duration of the behavioral data collection is too brief, some behaviors may be missed and the behavioral patterns of the target monkeys cannot be fully under-

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stood. Therefore, establishing a standard behavior collection protocol is an effective way of resolving these outstanding problems.

The DSM-5-TR (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, March 2022) defines ASD as characterized by the core symptoms of impaired social interactions, restricted interests, and repetitive behaviors (RRBs). Although the above five models all examine two of the core symptoms of ASD, namely social interactions and repetitive behaviors, they ignore the restricted interests in RRB, an important specific phenotype of ASD that may only exist in ASD patients. Additionally, even for the same social interactions, there were significant differences in the specific types of behaviors included for analysis, the active and passive analysis of social behaviors, and the inclusion criteria used by different groups. For example, regarding the specific social behaviors analyzed, Qiu's team (Liu et al., 2016) examined social contact and social grooming; Ji's team (Chen et al., 2017) evaluated social play, sharing toys, social grooming, and social proximity (within an arm's length); our laboratory (Wu et al., 2021) added social submission, such as screaming, crouching, running, lip-smacking, presenting mount and grimacing to the work of Ji's team; and Feng's team (Zhou et al., 2019) included expressions such as chasing, running, social following, social play, aggression, presenting mount, anogenital stimulation, social grooming, and mounting. Regarding the active and passive aspects of social behaviors, Ji's team (Chen et al., 2017) and our team (Wu et al., 2021) only observed target model monkeys actively initiating social behaviors, while Li's team (Tu et al., 2019) observed both active and passive social interactions. Regarding the inclusion criteria for social behaviors, Qiu's team (Liu et al., 2016) specified that social approach behaviors lasted at least 3 seconds before recording, while Feng, Ji, Li's, and our team adopted the principle of recording as soon as they occur (Chen et al., 2017; Tu et al., 2019; Zhou et al., 2019; Wu et al., 2021). These differences in assessment criteria create challenges in interpreting results and make it difficult for readers to determine whether discrepancies between different groups are due to actual biological differences or methodological variations. Consequently, many findings from monkey-based ASD studies are not directly comparable and are difficult to replicate, hindering the establishment, validation, and application of monkey ASD models.

After reviewing the differences among various team behavior assessment systems and the problems they caused, we proposed the following solutions. In terms of data collection, based on the facts that the model monkeys are generally cage-reared in standardized facilities and that the clinical diagnosis was focused on the individual patient's behavior, to obtain objective and continuous model monkey behavioral data that are suitable for the next analysis, we employed focal animal sampling (Altmann 1974; Lutz et al., 2022) using focused video tracking, where the recording camera is fixed on a specific target monkey to continuously track and record its behavior and surrounding environment. Additionally, we reviewed a large number of monkey daily behavior studies and integrated our extensive experience in monkey behavioral observations to establish a set of standardized and detailed behavioral data collection protocols tailored to different monkey housing environments, including single-cage housing and social group housing. In terms of ethogram formulation, we also referred to a large quantity of classic behavioral research literature and combined our own long-term behavioral observation research experience.

We first classified monkey daily behaviors into either individual or social behaviors. Monkeys housed in single cages were analyzed for individual behaviors, such as self-grooming and exploratory behaviors. For monkeys housed in social groups, we analyzed individual behaviors as well as social interactions, including social grooming, play, proximity, and contact. In the process of developing the ethogram, adhering to the principle that the definitions of the listed behaviors should not overlap with one other, we included as comprehensive a list of behavior types as possible, which is very important in gene-behavioral phenotype studies and can provide a sufficiently broad foundation for the development of sub-ethograms for various diseases. For individual behaviors, after excluding cases where the behaviors were the same but named differently, our ethogram listed all observable and measurable basic behaviors from the reviewed literature and defined them as basic individual behaviors. For social behaviors, we summarized and organized all behaviors related to social interactions reported in the literature to obtain basic social behaviors, the definitions of which did not overlap.

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Next, clear and explicit descriptions and inclusion criteria were provided for all basic behaviors in both categories. To facilitate a better analysis of the functional aspects of the behaviors, we grouped functionally similar basic behaviors into one composite behavior. To prevent confusion, once a basic behavior had been categorized into one composite behavior, it could not be categorized into other composite behaviors. Finally, we defined and gave specific names to the composite behaviors. This approach effectively addressed two major issues present in current behavior ethograms: inconsistencies in definitions, behavior types, behavior manifestations, and inclusion criteria for behaviors with the same name, and the use of different names for fundamentally the same behavior. Additionally, we included one to three typical video clips for each basic behavior in our ethogram, ensuring a clear and accurate understanding of the basic daily monkey behaviors by visualizing each behavior.

2 Methods

To address the significant heterogeneity of collection protocols and behavior ethograms in current behavioral studies of model monkeys, we developed standardized protocols for daily behavioral data collection and formulated a comprehensive daily behavior ethogram, forming a daily behavioral data collection and analysis system for both rhesus and cynomolgus monkeys (Fig. 1).

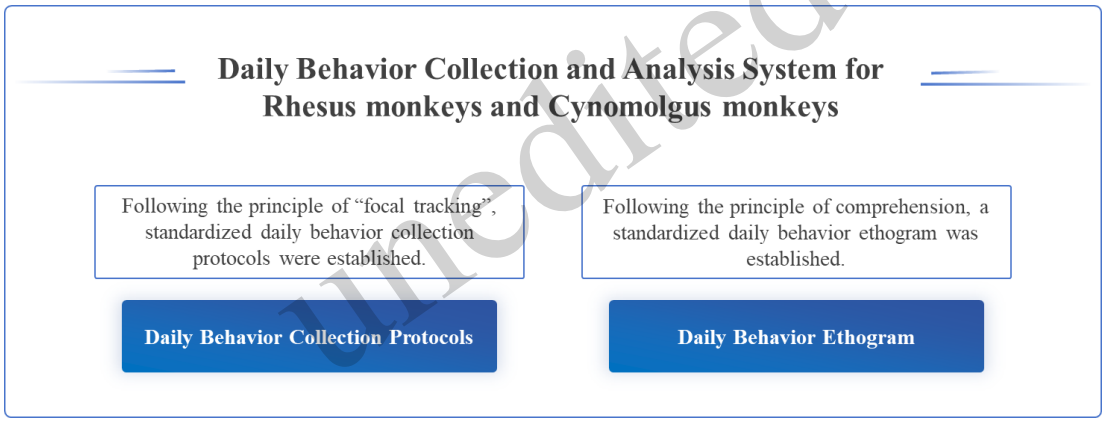

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Fig. 1 Composition of daily behavior collection and analysis system for rhesus monkeys and cynomolgus monkeys. Daily behavior collection and analysis system for rhesus monkeys and cynomolgus monkeys consists of two parts. 1) daily behavior collection protocols, which follows the principle of "focal tracking" to continuously collect monkey behavioral data; 2) a comprehensive monkey daily behavior ethogram.

2.1 Establishment of behavioral data collection protocols for rhesus and cynomolgus monkeys

Objective and standardized behavioral data collection protocols are essential for behavioral research. In daily behavioral studies, it is necessary to conduct comprehensive and continuous behavioral observations of target monkeys. Comprehensive observations involve recording all observable behaviors, while continuous observations mean uninterrupted recording over a certain duration. To achieve this, focal animal sampling (Altmann, 1974; Lutz, et al., 2022) was adopted here. This approach involves using a camera to continuously record all behaviors exhibited by an animal over a set period, providing a complete dataset for subsequent behavioral assessments. In addition to the behavioral data collection method, various other factors, such as living environment, recorders, recording time, and duration of recording, may impact monkey behaviors. For acquiring objective behavioral data, the authors reviewed numerous papers on behavioral studies of rhesus and cynomolgus monkeys. In conjunction with our long-term experiences, the influences on target monkeys of environment, recorders, recording time, and recording duration were considered for formulating precise mon-

key daily behavioral data collecting protocols. This ensured that the behavioral data from different research teams would be directly comparable. Table 1 summarizes the key factors influencing behavioral data collection and the corresponding countermeasures we developed.

Table 1 Important factors influencing the collection of rhesus and cynomolgus monkeys' behavioral data and corresponding countermeasures

Factors	Possible influencers	Countermeasures
Living habitat	Familiar habitats conducive to observing realistic daily monkey behavior. Collecting behavior during sudden changes in living habitat affected the accuracy of behavioral data (Fernström et al., 2008)	Consulting the literature and combining the experiences of keepers to recommend a minimum duration for which monkeys should reside in their current environment before behavioral data collection to ensure the data reflect behaviors in a familiar setting
Monkey markers	Marking is critical, especially for monkeys, forming the basis for identifying and tracking by video recorders	Providing guidelines for marking monkeys in group cages
Video recorders	During behavior recording, inappropriate video recorder actions interfered with monkey behaviors (Iredale et al., 2010)	Specifying specific details regarding gross appearance, distance from cages, range of movement, and noise level of video recorders to minimize any behavioral disturbance
Specific time/duration of behavioral data collection	Monkey behavior may be influenced by specific timing of collection (days & timepoints) with differential collection timepoints leading to great differences in behavioral data. Regarding duration, insufficient collection days and recording length enhanced chance errors, leading to failure to fully understand monkey behavioral patterns	Consulting the guideline for model construction and providing recommendations for specific timing and duration of behavioral data collection to minimize errors
Distance of video equipment from cages	Video equipment too close to cages interferes with behaviors and misses surroundings; too distant compromises video clarity	Recommending optimal distances between video equipment and monkey cages
Screen coverage of target monkeys	Screen coverage of target monkey should be adjusted to changes in behavioral states. For example, when a monkey is sprinting quickly, a high screen percentage is not ideal for tracking its trajectory	Screen coverage is assigned for different living habitats and various behavioral states of target monkeys.
Clarity of recorded videos	Video clarity directly impacts an analyst's capability to identify target monkey and accurately judge its behaviors (Balzamo et al., 1998)	Establishing clear requirements for video clarity to ensure high-quality behavioral recordings

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2.2 Establishment of daily behavior ethogram for rhesus and cynomolgus monkeys

After collecting behavioral data, the next step is analyzing behavioral data. According to the tradition of clinical symptom evaluation in monkey models of brain diseases, it is imperative to establish an ethogram for behavioral evaluation of monkey models. First, the ethogram must be comprehensive, encompassing as many observable and measurable monkey behaviors as possible. Second, it should be standardized—each behavior should be clearly defined so that there is no overlap among various behaviors. The manifestations and inclusion criteria of each behavior are definitely specified. To enhance the intuitive understanding of the ethogram, especially for inexperienced observers, one to three typical video clips are assigned for each basic behavior. These requirements are listed as basic principles for the ethogram of daily monkey behavior. To fulfill the requirement of comprehensiveness, we searched PubMed and selected 53 pieces of literature published between 1977 and 2024 based on the criteria that the article must: (1) focus on a daily behavior study; (2) must involve behavioral research on rhesus monkeys or cynomolgus monkeys; (3) contain a clear, explicit behavioral definition or description of its manifestations; (4) have unique features, i.e., if the ethograms used in two articles are highly similar, they will not be included together. After excluding instances where the same behaviors were named differently, all definitively non-overlapped behaviors directly observed and measured from the literature were designated as basic behaviors. To facilitate behavioral analysis, basic behaviors with similar functions were treated as composite behaviors. To maintain clear boundaries between different composite behaviors, each basic behavior was categorized under only one composite behavior. Then, clear description and inclusion criteria were applied for each basic behavior based on the literature and our long-term experience in behavioral analysis. Both a name and a definition were provided for each composite behavior. The literature for determining basic and composite behaviors in the ethogram is listed in Table S1. To enhance the intuitive understanding of the ethogram, especially for inexperienced observers, one to three typical video clips were prepared for each basic behavior. Six experienced observers sorted through 420 behavioral videos from 70 monkeys (six videos per monkey, 30-min clip) from our previous studies over a period of 1 year. Each observer screened and selected typical clips corresponding to each basic behavior. Finally, the most representative clips for each basic behavior were assigned as standard references. Finally, the Monkey Daily Behavior Ethogram (MDBE) was successfully established. To establish a core symptom assessment ethogram suitable for ASD monkey modeling, the authors consulted the DSM-5-TR, a globally authoritative tool for classifying and diagnosing mental disorders based on the core symptoms of ASD—social interaction dysfunctions and restricted interests and repetitive behaviors. These corresponded to three basic behaviors of MDBE, namely social interactions, stereotyped behaviors, and restricted interests. Through extracting and combining these behaviors of MDBE, the authors developed a specialized ethogram for evaluating the core symptoms of ASD in monkeys named the ASD Monkey Core Behavior Ethogram (MCBE-ASD).

2.3 Specific applications of MDBE and MCBE-ASD

To carry out a valid analysis of behavioral phenotypes of model monkeys with the above ethograms, a detailed description of how to use MDBE and MCBE-ASD is provided in the results section. To demonstrate the application of ethograms, three experienced behavior observers analyzed 30-minute video clips from three monkeys living in a social group using both MDBE and MCBE-ASD. The results of this analysis are presented in Section 3.3. The consistency of the observations was also examined. In addition, in order to demonstrate the differences in behavioral patterns among individual monkeys, we list six behaviors with the longest durations from each of three monkeys for comparison.

3 Results

We established a comprehensive and standardized system for collecting and analyzing the daily behavioral

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data of monkeys based on a detailed literature review and our long-term experience observing the behavior of rhesus and cynomolgus monkeys. The system includes daily monkey behavioral data collection protocols and daily monkey behavior ethograms.

3.1 Rhesus and cynomolgus monkeys' daily behavioral data collection protocols

The data collection protocol for monkey behavior employs focal animal sampling, focusing on a specific target monkey and continuously video-recording all behaviors over a set period. Standardized behavioral data collection protocols tailored for both single-cage and social groups were established. Measures for minimizing environmental impact, the presence of video recorders, and the timing/duration of recording were implemented.

3.1.1 Single-cage monkey behavioral data collection protocol

In single-cage housing, a monkey lives alone in a cage (usually 0.90 m×0.90 m×0.90 m), resulting in a limited locomotion range. As such, the camera can be placed on a tripod in front of the cage, with the operator only needing to operate the camera at the beginning and end of the recording session, and there is no need for the operator to track the monkey during the video recording (Fig. 2a). Details on the daily behavioral data collection protocols for single-cage monkeys are shown in Table 3.

3.1.2 Social group monkey behavioral data collection protocol

In a social group setting, several monkeys, usually 4–8, are housed together in a large enclosure that connects an indoor compartment (2.61 m×2.46 m×2.58 m) and an outdoor iron cage (2.67 m×2.66 m×2.67 m). The social group monkeys have a large range of locomotion, so placing a camera directly in front of the cage is insufficient for capturing the full range of movement trajectories. Therefore, the recording equipment needs to be adjusted during the video recording to focus on the movement trajectories of the target monkey and to record its social interactions (the monkeys interacting with the target monkey should be visible on the recording screen) (Fig. 2b). Details on behavioral data collection protocols for group-housed monkeys are summarized in Table 2.

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Fig. 2 Monkey daily behavioral data collection protocols. (a) Behavioral data collection set-up for a single-cage monkey. (b) An example of behavior recording of a single-cage monkey. (c) Behavioral data collection set-up for monkeys in a social group. (d) An example of behavior recording of a target monkey in a social group.

Table 2 Behavioral data collection protocols for single-cage and group-housed rhesus and cynomolgus monkeys

Factors	Common requirements for single-cage and social group monkeys	
Requirements of living habitat	Based on the literature on monkey model construction (Zhang et al., 2016; Galvão-Coelho et al., 2017; Lutz C K 2018; da Silva et al., 2019; Yin et al., 2020) combined with the experience of professional keepers, and considering the target monkey's diet, sleep, activity level, and interaction with other monkeys (group-housed monkeys), it is suggested that target monkeys should have lived in their current habitat for at least one month prior to the start of data collection.	
Requirements for video recorders	(i) Monkeys must pass a medical examination confirming they are free from infectious diseases that could impact their health; (ii) standard dress code; (iii) no loud noise during the recording and avoid large movements.	
Pre-recording requirements	Before formal recording, the video recorders should conduct a pre-recording session for at least 5 days to: (i) acclimate monkeys to the recording equipment and personnel, and (ii) familiarize themselves with the recording process.	
Recording time and duration	For recording time, to capture a complete behavioral pattern of the target monkey, it is recommended that video recording should be conducted in both the morning and afternoon. To reduce the impact of other factors, times when keepers are feeding and cleaning monkey cages and veterinarians are conducting examinations should be avoided (Griffis et al., 2013). In our experience, behavioral data are usually not collected within 30 minutes after feeding, cage cleaning, or veterinary examination. Referring to previous monkey model research literature, for recording duration, it is recommended to record for at least 30 min twice a day, once in the morning and once in the afternoon (Feng et al., 2011; Griffis, et al., 2013; Camus et al., 2014; Hage, et al., 2014; Truelove et al., 2017; Stewart et al., 2023). For behavioral observation duration, it is recommended that recordings should be conducted for seven consecutive days or more for each monkey (Qin et al., 2015; Wu, et al., 2021). During a behavioral data collection cycle, one can choose to record the time periods of 9:00–9:30 and 14:00–14:30 on the first day, 9:30–10:00 and 14:30–15:00 on the second day, and so on, trying to cover the behavior of the target monkey during all time periods.	
Clarity requirements for videos	The video should provide a clear visual image, and the standard is: the monkey's detailed movements should be clearly visible in the video file. Image quality should be based on the specific conditions of the monkey's surroundings, with a minimum of High Quality (HQ) and, if necessary, Full High Definition (FHD).	
Factors	Different requirements for single-cage and social group monkeys	
	Single cage	Social group
Marking for target monkeys	No marking is required for single-cage monkeys.	Target monkeys can be marked for identification by shaving, which should be trackable by video recorder. Possible shaving sites include back of the waist, back of the neck, limbs, or other body parts. If the monkey is shedding, the marking site should be chosen to avoid areas where shedding occurs.
Recording protocol	To begin recording, the video recorder first sets up the camera on a tripod and displays a sheet of A4 paper in front of camera showing the target monkey's identity and location. Once the recording time has elapsed, the video recorder turns off the camera and removes the equipment.	At the beginning of the recording, the recorder should provide a clear description of the date, time, weather conditions, target monkey's identification number, shaving mark characteristics and location of target monkey in video. During recording, a recorder should (i) describe the monkey's movement trajectory and behavior for subsequent analysis and (ii) note any changes in external environment (including but not limited to: weather conditions such as thunder and rain, events such as people waking past the cage). The recorder should keep their

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		voice clear but not interfere with behavior.
Distance of video equipment from the monkey cages	To avoid disturbing behaviors, a distance of at least 1.5 m should be maintained between video equipment and front of the cage.	To ensure video recording clarity and avoid interference with the target monkey, a distance of at least 2 m should be maintained between video equipment and front of the cage.
Screen percentage of the target monkeys	To capture all behaviors, the recording screen should cover the entire target monkey's cage. To avoid visual interference, try not to capture other monkey cages on screen, see Fig. 2b.	Initially, the recording should cover the entire cage, then adjust to focus on the target monkey. During recording, screen coverage of the target monkey should be adjusted according to its behavior. The maximum percentage requirement is to ensure that target monkey is completely in screen (recommended maximum percentage is 33%); the minimum percentage is to ensure that the detailed movements of the target monkey can be seen clearly (the recommended minimum percentage is 11%). When the target monkey's movement range is large, adjust the focus to make the target monkey's proportion smaller to facilitate tracking of its activity trajectories; when the target monkey's activity range is small or has no position movement, increase its proportion. When the target monkey interacts with other monkeys, include peer in the screen, see Fig. 2d.
Recording of social interactions	Single-caged target monkeys are not involved in social interactions.	To ensure valid recording of social interactions, on the basis of focusing on the target monkey, the monkeys interacting with the target monkey are also required to be on the recording screen.

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3.2 Daily behavior assessment ethogram (MDBE) for rhesus and cynomolgus monkeys

Individual behaviors consist of 47 basic behaviors that are directly observable and measurable in rhesus and cynomolgus monkeys, with no overlap in definition. These basic behaviors were classified, based on functional similarities, into the following five composite behaviors: voluntary locomotion, exploration, stereotyped, response to external stimuli, and foraging and eating behavior. Social behaviors encompassed 23 basic behaviors. Among them, 19 behaviors were grouped as social interactions (e.g., sexual behavior, social grooming, social play, social contact, social proximity, and social following). The remaining four basic behaviors, namely social withdrawal, sitting alone, non-social, and rejected mount, are the opposite of social interactions, and their assessment is also very important for the target monkey's social interactions.

To assist users in understanding the targeted monkey behaviors in MDBE, we provided the names of the basic behaviors (first column from the left in Table 3), their specific descriptions (second column), and inclusion criteria (third column). We also listed the names of composite behaviors generated by combining functionally similar basic behaviors (fifth column), along with their definitions (sixth column). See Table 3 for details. Additionally, we included one to three typical behavior videos for each basic behavior after careful screening, with their corresponding indices listed in Table 3 (fourth column). These video clips are placed in the Supplementary Materials.

Next, based on MDBE and by referring to DSM-5-TR for the core clinic symptoms of ASD, which include social interactions, stereotyped behaviors, and restricted interests, we combined them into an ASD core clinical behaviors assessment ethogram suitable for ASD model validation of rhesus and cynomolgus monkeys, referred to as MCBE-ASD (refer to Table 4 for details).

3.3 Uses and applications of MCBE-ASD

3.3.1 Uses of ethograms

Using the above ethograms, we can quantitatively assess the daily behavioral phenotype of the model monkey by measuring the timing and frequency of the behavior occurrence. Specifically, experienced behavior analysts strictly adhere to the names, definitions, specific manifestations, and inclusion criteria provided in the ethograms, then judge all the behaviors of the target monkey displayed in a video clip, accurately record the start and end times of each behavior, and finally calculate the frequency and duration of the behavior. The specific process of behavioral analysis is as follows: First, the analyst selects a video file for analysis and records the target monkey number, video name, total video duration, analysis time, and analyst name. The video file is then imported into video software, such as Adobe Premiere Pro software (Adobe Inc., USA), and the analyst identifies and records all displayed behaviors from the beginning to the end of the video.

Taking the stereotyped behavior of digit-suck as an example, when Monkey 1 in the video displays digit-sucking behavior, the analyst manually pauses the video to record the start time (e.g., 15:00) and resumes play. When the digit-suck behavior stops, the recording is paused again to record the end time (e.g., 15:11). The interval between these two time points is the duration of Monkey 1's digit-suck behavior (i.e., 11 seconds). This behavior may occur several times within the video. The total duration of Monkey 1's digit-sucking behavior in the video is calculated by summing all time periods during which the behavior occurred, while the frequency of the behavior is the number of episodes. The duration and frequency of all digit-suck behaviors of Monkey 1 in the video clip are added up and then divided by the total duration of the video to obtain the duration and frequency per unit time, expressed in seconds per hour (s/h) and frequency per hour (f/h), respectively. The same analysis procedure is applied to other behaviors in the ethogram.

Sometimes, the target monkey performs two (or more) behaviors at the same time; the specific start and end times of each behavior must be recorded in detail. For example, the target monkey started digit-sucking at 5′ 0″, then started to make contact with another monkey at 5′ 5″, stopped digit-sucking at 5′ 10″, and the other monkey walked away and stopped the contact at 5′ 15″. This situation should be recorded as follows: digit-suck: 5′ 0″–5′ 10″; social contact: 5′ 5″–5′ 15″. To minimize subjective influence, the "double-blind principle" should be strictly followed when using the ethograms for behavioral analysis, ensuring the analyst is unaware of any

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specific information about the subjects. Additionally, it is recommended that the same video file be analyzed simultaneously by three experienced analysts. When the consistency between their analyses is ≥ 95%, the results from each analyst can be averaged to obtain the final behavioral data.

3.3.2 Results of using MDBE and MCBE-ASD

To test the repeatability of the behavioral analysis system described above, three experienced analysts independently analyzed three 30-minute video files of three group-housed monkeys using MDBE and MCBE-ASD, respectively. The results showed that the consistency of their analysis results for various behaviors was between 95.04% and 100%, meeting the requirement of 95% consistency. Subsequently, the average duration (s/h) and frequency (f/h) of each behavior were calculated for the three monkeys. Tables 5, 6, S2 and S3 summarize the duration and frequency of the 70 basic behaviors and six comprehensive behaviors of the three monkeys, respectively. Tables 7 and S4 summarizes the duration and frequency of the three core ASD behaviors. For the duration and frequency of each behavior, we list the specific values of each analyst (A, B, and C) as well as their consistency and the average of their results (data are shown as mean ± SEM). Overall, the results showed that MDBE and MCBE-ASD enabled efficient monkey daily behavior quantification analysis. Then, by comparing the duration of various behaviors of three rhesus monkeys, we found that different individuals displayed different behavioral patterns. For example, Monkey 1 displayed the most stereotyped behaviors, while Monkey 2 and Monkey 3 spent more time on voluntary locomotion and social interactions (Fig. S1).

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Table 3 Monkey Daily Behavior Ethogram (MDBE)

Basic behavior name	Basic behavior description	Basic behavior inclusion criteria	Index of basic behavior video clips	Composite behavior name and contents	Composite behavior definition
Walking	Moving slowly using all four limbs alternately	/	1-1	Voluntary locomotion (including walking, climbing, running, and jumping)	Target monkey initiates behavior autonomously, generating positional changes.
Climbing	Climbing along cage or crawls on ground using all four limbs	/	2-1		
Running	Moving rapidly using all four limbs alternately	/	3-1		
Jumping	Utilizing force through its hind limbs or all four limbs simultaneously to elevate its body from the ground, including vertical jumps and leaps to various locations	/	4-1		
Hanging	Hanging from cage top or side, with all four limbs off ground and no positional changes	/	5-1, 5-2	/	/
Standing	Standing on all four limbs or hind limbs without positional changes	/	6-1, 6-2	/	/
Voluntary vocalization	Making a sound in the absence of an obvious stimulus	/	7-1	/	/
Self-grooming	Using mouth, tongue, hands, and feet for self-grooming. Common forms include: 1) grooming fur with hands or feet; 2) licking fur with mouth and tongue.	/	8-1, 8-2	/	/
Scratching	Using hands or feet to scratch or rub parts of body, such as head, arms, legs, or torso	/	9-1, 9-2	/	/
Hair-pull	Pulling hairs from oneself or others softly, as distinguished from plucking in self-injury, which is usually followed by hair-eat	/	10-1, 10-2, 10-3	/	/
Hair-eat	Chewing or ingesting hair	/		/	/

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Abnormal mouth movements	Moving mouth, lips or tongue repeatedly, not associated with eating or containing objects in mouth	/	11-1	/	/
Huddling	Adopting a posture with body bent, limbs and head tightly tucked, head at or below shoulders with eyes usually open	/	12-1	/	/
Rubbing ground	Rubbing hand back and forth on floor or another platform	/	13-1, 13-2	/	/
Tactile manipulation exploration	Engaging in physical manipulation of the cage or other objects in the environment, excluding food, through actions such as picking or touching, utilizing hands or feet	Specific objects are documented	14-1, 14-2	Exploration behavior (including tactile manipulation exploration and oral and olfactory exploration)	Target monkey engages in oral, olfactory, or tactile manipulation of objects (excluding food) within its environment using its hands, feet, mouth, and tongue
Oral and olfactory exploration	Exploring (licks & sniffs) cage or other objects using mouth or tongue	Specific objects are documented	15-1, 15-2		
Restricted interests	Displaying unusual, focused, and narrow interest in specific objects or environments (excluding food) while lacking interest in other objects/domains	Specific objects are documented	16-1	/	/
Swinging	Hanging and swinging back and forth at cage top or side	Must be repeated at least twice	17-1, 17-2	Stereotyped behaviors (including swinging, pacing, rocking, back-flipping /body-flipping, spinning, bouncing, digit-sucking, self-grasping/clasping, and saluting)	Target monkey exhibits repetitive, fixed movements or behavioral patterns that usually lack a clear purpose or function
Pacing	Walks/climbs/jumps back and forth along a repetitive route, including mechanically circling in cage	Route should be repeated at least twice (back and forth is considered once). Circling must involve a minimum of two rotations	18-1, 18-2, 18-3		
Rocking	Keeping its feet still while swaying its upper body back and forth	Must be repeated at least twice	19-1, 19-2		

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Back-flipping/Body-flipping	Repeatedly performing the same vertical body flip motion	Must be repeated at least twice	/		
Spinning	Repeatedly spinning or twirling around itself in a horizontal direction	Must be repeated at least twice	/		
Bouncing	Repeatedly bouncing up and down in the same position	Must be repeated at least twice	20-1		
Digit-sucking	Continuously sucking its fingers, toes, or other body parts	Must last for at least 3 seconds	21-1		
Self-grasping/clasping	Continuously grasping or holding its own limbs, tail, or other body parts	Must last for at least 3 seconds	22-1		
Saluting	Repeatedly placing its hand or finger over its eyes without causing injury	Must be repeated at least twice	23-1		
Other stereotyped behaviors	Displaying other repetitive and fixed movements or behavioral patterns, distinct from previously delineated behavioral categories	/	/		
Self-injurious behaviors	Intentionally injuring its own body, commonly in the form of hair plucking, self-biting, self-hitting and head/body banging, with severe cases resulting in punctured or torn skin	/	24-1, 24-2	/	/
Teeth-baring	Opening mouth widely in a threatening manner and displaying its teeth	/	25-1	Response to external stimuli (including teeth-baring, grimacing, tail-crooking, lip-smacking, freezing, spasming, cage-shaking, attacking people/objects, present mount, positional movement, vocalization, yawning, and stamping)	Target monkey responds to external stimuli, including, but not limited to, people passing by or talking loudly near them, keepers entering the monkey cage next door, stimuli from monkey next door, sound of thunder, etc.
Grimacing	Displaying an exaggerated grin and showing its teeth	/	/		
Tail-crooking	Adopting a posture characterized by alert elevation of its tail	/	26-1		
Lip-smacking	Rapid twitching of mouth	/	27-1		
Freezing	Sitting or crouching in a stiff manner, remaining motionless for at least 3 seconds	Must last for at least 3 seconds	/		

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Spasming	Violent shaking or spasmodic movements of its body	/	/		
Cage-shaking	Violently shaking cage, steel bars, rails, or swing	/	28-1		
Attacking people/objects	Target monkey exhibits hostility or aggression toward other monkeys/organisms or objects	/	/		
Present mount	Exhibiting hip and tail lifting postures in non-sexually related contexts	/	/		
Positional movement	Engaging in positional change behaviors such as walking, climbing, running, and jumping, in response to external stimuli	/	29-1, 29-2		
Vocalization	Emitting vocalizations, inclusive of screams, as a response to external stimuli (e.g., keeper passing by, commotion in an adjacent monkey cage)	/	30-1, 30-2, 30-3		
Yawning	Opening mouth widely and inhales, then slowly exhales	/	31-1, 31-2, 31-3		
Stamping	Jumping or trotting rhythmically on its feet and then lands quickly, accompanied by tensing and stiffening of limbs	/	/		
Other responses to external stimuli	Displaying responses to external stimuli in addition to above categories	/	/		
Foraging	Foraging for food in the surroundings	/	32-1	Foraging and eating, drinking (including foraging, eating food, and drinking water)	Target monkey acquires and consumes food and water in the environment to fulfill physiological needs
Eating food	Ingesting food	/	33-1, 33-2		
Drinking water	Drinking water from tap or other sources (e.g., puddles)	/	/		
Resting	Displaying recumbent behaviors such as laying, reclining, sprawling, and sitting to meet rest and sleep needs	/	34-1	/	/

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Masturbatory behavior	Stimulates its genitals with its hands or other body parts	/	/	/	/
Sexual behavior	Sexual intercourse between target monkey and monkey of the opposite sex	/	/	Social interactions [a] (including sexual behavior, mounting, failed mount, anogenital stimulation, grooming solicitation, social grooming, social play, aggressive behavior, submissive behavior, take over, walking side-by-side, social contact, ventral contact, social proximity, social following, passing contact, and bump)	Social communication and interaction between two or more monkeys, including social play, grooming, contact, proximity, following, etc.
Mounting	Mounting the hips of another monkey of the same sex	/	35-1		
Failed mount	Trying to mount the hips of another monkey and failing		36-1		
Anogenital stimulation	Stimulating genitals of another monkey with its hands or other body parts	/	/		
Grooming solicitation	Presenting body parts (excluding hip) to peers, e.g., back, chest or limbs, etc., and waiting to be groomed		37-1, 37-2		
Social grooming	Touching another monkey's fur, involving picking, scraping, spreading, mouth picking, and licking	/	38-1, 38-2, 38-3		
Social play	Engaging in common forms of play, including fighting, rolling, slapping, pulling, wrestling, pushing, chasing, grasping, and softly biting, without obvious hostility or outcome	/	39-1, 39-2		
Mild aggression	Engaging in common forms of mildly aggressive behavior, such as eye threats, lip smacking [c], half-open mouth, open-mouthed bared-teeth scream, and displacement [b]	/	40-1		
Extreme aggression	Engaging in common forms of extremely aggressive behavior, such as chasing, biting, hitting, and grabbing	/	41-1		
Mild submission	Engaging in common forms of mildly submissive behavior, such as lip-smacking [c], grimacing, freezing, and yielding position to another monkey [b]	/	42-1		

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Extreme submission	Engaging in common forms of extremely submissive behavior, such as screaming, crouching, and running	/	43-1, 43-2		
Take over	Snatching objects (including food) from peers		44-1, 44-2		
Walking side-by-side	Walking side-by-side with another monkey, usually with the upper limbs resting on each other, similar to a "two-person tripod"	/	45-1, 45-2		
Social contact	Except engaging in social play, social conflict, social grooming, side-by-side walking, sexual behavior and sex-related behavior, initiates skin/hair contact with another monkey	/	46-1		
Ventral contact	Using abdomen to make contact with body parts of another monkey, commonly ventral to ventral or ventral to dorsal	/	47-1, 47-2		
Social proximity	Staying within an arm’s length of another monkey, but not in contact	/	48-1, 48-2		
Social following	Following another monkey, maintaining a relative position within an arm's length	/	49-1, 49-2		
Passing contact	Briefly contacting other monkeys with body (except for mouth/hands) during positional movements, but without stopping moving		50-1, 50-2		
Bump	Make physical contact (except for mouth/hands) with peers during positional movements, then stop moving	/	/		
Social withdrawal	Moving away or ignores other monkeys' social intentions, including contact, grooming, playing, or threatening	/	51-1	/	/
Rejected mount	Refusing to be mounted by another monkey		52-1	/	/

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Sitting alone	Staying alone in one place, showing no other behaviors and with no other monkeys within an arm's length	/	53-1	/	/
Non-social	Performing certain behaviors alone, such as voluntary locomotion, without social interactions with peers	/	54-1	/	/

a. All basic behaviors of social interactions can be actively initiated or passively accepted by target monkey.

b. Displacement refers to occupying the original position or space of other monkeys, generally initiated by monkeys of high status in the social group. Yielding position to another monkey refers to giving up its position or space to another monkey, usually initiated by monkeys of low status in the social group.

c. Appearance of lip smacking by target monkey sometimes indicates threat and sometimes submission, which needs to be judged within the context of the specific social situation and based on body posture and facial expression of target monkey.

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Table 4 Monkey Core ASD Behavior Ethogram (MCBE-ASD)

Core behavior name	Core behavior contents
Social interactions	Sexual behavior, mounting, failed mount, anogenital stimulation, grooming solicitation, social grooming, social play, aggressive behavior, submissive behavior, take over, walking side-by-side, social contact, ventral contact, social proximity, social following, passing contact, and bump
Stereotyped behaviors	Swinging, pacing, rocking, back-flipping/body-flipping, spinning, bouncing, digit-sucking, self-grasping/clasping, and saluting
Restricted interests	/

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Table 5 Results of basic behaviors of three monkeys in a social group using MDBE

Name of basic behavior		Monkey 1 (Female, 3.43 years old)-Video 1		Monkey 2 (Female, 3.39 years old)-Video 2		Monkey 3 (Male, 3.41years old)-Video 3	
		Consistency（%）	mean ± SEM	Consistency（%）	mean ± SEM	Consistency（%）	mean ± SEM
Walking	Duration (s/h)	99.54	428.67 ± 1.15	97.65	825.75 ± 11.20	98.78	515.59 ± 3.64
	Frequency (f/h)	98.36	121.06 ± 1.15	99.44	205.10 ± 0.67	98.66	169.46 ± 1.31
Climbing	Duration (s/h)	97.56	81.37 ± 1.15	97.96	293.67 ± 3.46	99.34	346.13 ± 1.31
	Frequency (f/h)	96.84	36.38 ± 0.66	97.37	75.92 ± 1.15	98.92	104.69 ± 0.65
Running	Duration (s/h)	95.06	23.15 ± 0.66	100.00	5.99 ± 0.00	95.32	24.21 ± 0.65
	Frequency (f/h)	95.05	23.15 ± 0.66	100.00	5.99 ± 0.00	100.00	5.89 ± 0.00
Jumping	Duration (s/h)	98.91	104.52 ± 0.66	95.45	43.95 ± 1.15	100.00	9.81 ± 0.00
	Frequency (f/h)	96.97	65.49 ± 1.15	97.06	39.29 ± 0.67	100.00	7.85 ± 0.00
Hanging	Duration (s/h)	97.66	129.66 ± 1.75	98.39	123.86 ± 1.15	99.32	597.38 ± 2.36
	Frequency (f/h)	96.68	34.40 ± 0.66	95.44	25.30 ± 0.67	98.27	65.43 ± 0.65
Standing	Duration (s/h)	95.67	52.92 ± 1.32	95.44	25.30 ± 0.67	95.44	24.86 ± 0.65
	Frequency (f/h)	95.05	23.15 ± 0.66	100.00	7.99 ± 0.00	100.00	7.85 ± 0.00
Voluntary vocalization	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Self-grooming	Duration (s/h)	100.00	0.00 ± 0.00	100.00	11.99 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	2.00 ± 0.00	100.00	0.00 ± 0.00
Scratching	Duration (s/h)	97.75	50.94 ± 0.66	95.46	67.26 ± 1.76	100.00	13.74 ± 0.00
	Frequency (f/h)	95.05	23.15 ± 0.66	95.05	23.31 ± 0.67	100.00	5.89 ± 0.00
Hair pull	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Hair-eat	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00

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Abnormal mouth movements	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Huddling	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Rubbing ground	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Tactile manipulation exploration	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	95.45	24.87 ± 0.65
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	9.81 ± 0.00
Oral and olfactory exploration	Duration (s/h)	100.00	5.95 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	3.97 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Restricted interests	Duration (s/h)	98.67	86.00 ± 0.66	97.81	52.61 ± 0.67	98.29	66.09 ± 0.65
	Frequency (f/h)	95.05	23.15 ± 0.66	100.00	9.99 ± 0.00	4.68	24.21 ± 0.65
Swinging	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	3.93 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	1.96 ± 0.00
Pacing	Duration (s/h)	99.82	1 125.25 ± 1.15	99.14	134.52 ± 0.67	98.29	66.09 ± 0.65
	Frequency (f/h)	96.53	33.08 ± 0.66	100.00	5.99 ± 0.00	100.00	3.93 ± 0.00
Rocking	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Back-flipping/Body-flipping	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Spinning	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Bouncing	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Digit-sucking	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00

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Self-grasping/clasping	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Saluting	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Other stereotyped behaviors	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Self-injurious behavior	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Teeth-baring	Duration (s/h)	100.00	0.00 ± 0.00	95.44	25.31 ± 0.67	100.00	5.89 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	5.99 ± 0.00	100.00	3.93 ± 0.00
Grimacing	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Tail-crooking	Duration (s/h)	100.00	0.00 ± 0.00	97.56	81.91 ± 1.15	95.33	24.21 ± 0.65
	Frequency (f/h)	100.00	0.00 ± 0.00	96.06	29.30 ± 0.67	100.00	9.81 ± 0.00
Lip-smacking	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Freezing	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Spasming	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	1.96 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	1.96 ± 0.00
Cage-shaking	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Attacking people/objects	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Present mount	Duration (s/h)	100.00	0.00 ± 0.00	95.97	28.64 ± 0.67	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	9.99 ± 0.00	100.00	0.00 ± 0.00

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Positional movement	Duration (s/h)	100.00	0.00 ± 0.00	100.00	9.99 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	4.00 ± 0.00	100.00	0.00 ± 0.00
Vocalization	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Yawning	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Stamping	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Other responses to external stimuli	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Foraging	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	5.89 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	1.96 ± 0.00
Eating food	Duration (s/h)	97.46	44.98 ± 0.66	100.00	0.00 ± 0.00	99.40	499.24 ± 1.73
	Frequency (f/h)	100.00	7.94 ± 0.00	100.00	0.00 ± 0.00	98.03	57.58 ± 0.66
Drinking water	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Resting	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Masturbatory behavior	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Sexual behavior	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Mounting	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Failed mount	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00

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Anogenital stimulation	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Grooming solicitation	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Social grooming	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Social play	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Mild aggression	Duration (s/h)	100.00	0.00 ± 0.00	95.07	23.31 ± 0.66	100.00	5.89 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	5.99 ± 0.00	100.00	3.93 ± 0.00
Extreme aggression	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	95.04	22.90 ± 0.66
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	9.81 ± 0.00
Mild submission	Duration (s/h)	100.00	0.00 ± 0.00	95.07	23.31 ± 0.66	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	7.99 ± 0.00	100.00	0.00 ± 0.00
Extreme submission	Duration (s/h)	100.00	0.00 ± 0.00	100.00	4.00 ± 0.00	100.00	9.81 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	2.00 ± 0.00	100.00	3.93 ± 0.00
Take over	Duration (s/h)	100.00	1.98 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	1.98 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Walking side-by-side	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Social contact	Duration (s/h)	97.55	123.70 ± 1.75	95.18	133.19 ± 3.71	100.00	3.93 ± 0.00
	Frequency (f/h)	100.00	15.88 ± 0.00	95.31	24.64 ± 0.67	100.00	1.96 ± 0.00
Ventral contact	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Social proximity	Duration (s/h)	99.42	717.09 ± 2.39	99.59	736.52 ± 1.76	98.98	482.22 ± 2.85
	Frequency (f/h)	98.34	68.80 ± 0.66	97.14	69.92 ± 1.15	98.21	63.47 ± 0.66

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Social following	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Passing contact	Duration (s/h)	98.54	78.72 ± 0.66	98.05	118.53 ± 1.33	97.06	38.6 ± 0.65
	Frequency (f/h)	98.51	76.74 ± 0.66	96.82	72.59 ± 1.33	95.44	24.86 ± 0.65
Bump	Duration (s/h)	100.00	7.94 ± 0.00	98.00	99.89 ± 1.15	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	3.97 ± 0.00	97.99	57.27 ± 0.67	100.00	0.00 ± 0.00
Social withdrawal	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Rejected mount	Duration (s/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
	Frequency (f/h)	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00
Sitting alone	Duration (s/h)	99.61	298.34 ± 0.66	98.99	395.56 ± 2.31	99.48	374.92 ± 1.13
	Frequency (f/h)	95.78	27.12 ± 0.66	97.96	56.61 ± 0.67	97.07	38.61 ± 0.65
Non-social	Duration (s/h)	99.77	2670.56 ± 3.50	99.55	2461.27 ± 6.42	99.87	3036.64 ± 2.27
	Frequency (f/h)	99.97	3432.64 ± 0.66	99.88	3359.6 ± 2.40	100.00	3495.97 ± 0.00

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Table 6 Results of composite behaviors of three monkeys in a social group using MDBE

Name of composite behavior		Monkey 1 (Female, 3.43 years old)-Video 1		Monkey 2 (Female, 3.39 years old)-Video 2		Monkey 3 (Male, 3.41 years old)-Video 3	
		Consistency（%）	mean ± SEM	Consistency（%）	mean ± SEM	Consistency（%）	mean ± SEM
Voluntary locomotion	Duration (s/h)	99.22	637.71 ± 2.88	97.67	1169.37 ± 15.74	98.96	895.75 ± 5.36
	Frequency (f/h)	98.60	246.09 ± 1.98	99.07	326.30 ± 1.76	99.61	287.90 ± 0.65
Exploration behavior	Duration (s/h)	100.00	5.95 ± 0.00	100.00	0.00 ± 0.00	95.44	24.86 ± 0.65
	Frequency (f/h)	100.00	3.97 ± 0.00	100.00	0.00 ± 0.00	100.00	9.81 ± 0.00
Stereotyped behaviors	Duration (s/h)	99.82	1125.25 ± 1.15	99.14	134.52 ± 0.67	98.38	70.01 ± 0.66
	Frequency (f/h)	96.53	33.08 ± 0.66	100.00	5.99 ± 0.00	100.00	5.89 ± 0.00
Response to external stimuli	Duration (s/h)	100.00	0.00 ± 0.00	98.53	135.85 ± 1.15	96.47	32.06 ± 0.65
	Frequency (f/h)	100.00	0.00 ± 0.00	95.84	47.95 ± 1.15	100.00	15.70 ± 0.00
Foraging and eating, drinking	Duration (s/h)	97.45	44.98 ± 0.66	100.00	0.00 ± 0.00	99.41	505.13 ± 1.73
	Frequency (f/h)	100.00	7.94 ± 0.00	100.00	0.00 ± 0.00	98.10	59.54 ± 0.65
Social interactions	Duration (s/h)	99.35	929.44 ± 3.50	99.02	1138.73 ± 6.42	99.30	563.36 ± 2.27
	Frequency (f/h)	99.32	167.36 ± 0.66	98.27	240.4 ± 2.40	100.00	104.03 ± 0.00

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Table 7 Results of ASD core behaviors in three monkeys in a social group using MCBE-ASD

ASD core behavior name		Monkey 1 (Female, 3.43 years old)-Video 1		Monkey 2 (Female, 3.39 years old)-Video 2		Monkey 3 (Female, 3.41 years old)-Video 3	
		Consistency（%）	mean ± SEM	Consistency（%）	mean ± SEM	Consistency（%）	mean ± SEM
Social interactions	Duration (s/h)	99.35	929.44 ± 3.50	99.02	1138.73 ± 6.42	99.30	563.36 ± 2.27
	Frequency (f/h)	99.32	167.36 ± 0.66	98.27	240.4 ± 2.40	100.00	104.03 ± 0.00
Stereotyped behaviors	Duration (s/h)	99.82	1125.25 ± 1.15	99.14	134.52 ± 0.67	98.38	70.01 ± 0.66
	Frequency (f/h)	96.53	33.08 ± 0.66	100.00	5.99 ± 0.00	100.00	5.89 ± 0.00
Restricted interests	Duration (s/h)	98.67	86.00 ± 0.66	97.81	52.61 ± 0.67	98.29	66.09 ± 0.65
	Frequency (f/h)	95.05	23.15 ± 0.66	100.00	9.99 ± 0.00	95.32	24.21 ± 0.65

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4 Discussion

To solve the problem of heterogeneity in the evaluation of behavioral phenotypes in studies using monkey models of brain diseases, the present study established a standardized daily behavior collection and analysis system for rhesus and cynomolgus macaques, which are the non-human primates most commonly used for model development. This system was based on an extensive literature review and our long-term experience in monkey behavioral studies and was established on the principles of comprehensiveness and standardization, including behavioral data collection protocols and a daily behavior ethogram (MDBE). We demonstrated the specific application of MDBE in brain disease modeling validation using ASD as an example. That is, on the basis of MDBE, a sub-ethogram for the quantitative assessment of core clinical symptoms in the ASD monkey model (MCBE-ASD) validation was established according to the DSM-5-TR, a globally recognized authoritative tool for the classification and diagnosis of mental disorders. Subsequently, we used both MDBE and MCBE-ASD to analyze the behavioral video files of three socially housed monkeys to demonstrate their application. The data not only demonstrate the high reproducibility of the system but also show the differences in the behavioral patterns of these three individual rhesus monkeys.

The main advantages of our system are as follows. First, MDBE covers all observable and measurable daily behaviors of rhesus and cynomolgus monkeys that we found in the literature, including 47 basic individual behaviors and 23 basic social behaviors, covering the behavioral phenotypes of monkeys housed in both single cages and social groups. These behaviors can be used in juveniles, adolescents, adults, and elderly monkeys and apply to both females and males. For each basic behavior, MDBE provides a clear description and inclusion criteria. Therefore, in addition to its wide coverage, MDBE has the characteristics of standardization. Second, MDBE contains a hierarchy of basic behaviors and composite behaviors, which facilitates a better understanding and systematic analysis of the characteristics and patterns of the model monkey's daily behavior. In addition, for the basic behaviors in the MDBE, we included one to three typical video clips as references. This feature greatly enhances the user's understanding of rhesus and cynomolgus monkeys' daily behaviors, thereby improving behavioral analysis accuracy.

Of course, this system also has some limitations. First, it is only applicable to rhesus and cynomolgus monkeys. When extending the system to other macaque species, users need to carefully consider the variability of behavioral characteristics among different species. Secondly, considering the ethical restrictions and common cases in monkey brain disease modeling research, we only included the behaviors of juvenile, adolescent, adult, and aged monkeys, and not maternal or infant behaviors. In addition, although we have demonstrated the repeatability of the system, due to time limitations, we have not yet verified the long-term stability of the system. We will continue to follow up on this issue in the future. Additionally, 30 minutes was chosen as the focused recording time based on previous studies (Feng, et al., 2011; Griffis, et al., 2013; Camus, et al., 2014; Hage, et al., 2014; Qin et al., 2016; Truelove, et al., 2017; Tu, et al., 2019; Wu, et al., 2021; Stewart, et al., 2023) and our own experience. This is because 30 minutes can effectively capture most daily behaviors, avoiding the inability to fully understand the monkeys' behavioral patterns due to short recording time. At the same time, problems of data redundancy and recorder fatigue can be avoided. Although we have not yet conducted a systematic comparative study on the relationship between different recording times (e.g., 10/30/60 minutes) and behavioral acquisition effects, this is important research that we will conduct in the future.

It is important to note that the choice of behavioral data collection and analysis methods depends greatly on the purpose of the study. The goal of this study was to quantitatively analyze the daily behavioral phenotypes of brain disease model monkeys, which are usually housed in cages in good monkey facilities; therefore, we chose the method of focal sampling recording for collecting data on monkey behaviors. The advantage of focal sampling is that it can accurately record the frequency, duration, and order of occurrence of behaviors, thereby facilitating a detailed understanding of the behavioral patterns—especially complex behaviors—of the individuals of interest. However, there are drawbacks to this approach: sometimes, social interactions or responses to the environments are hard to explain because of the camera’s screen limitation. To alleviate this shortcoming,

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while tracking the activities of target monkeys, the recorder is required to adjust the camera to try to capture the surrounding individuals as much as possible. Simultaneously, a recorder should provide oral descriptions of the changes in the surrounding environment in the video, such as loud noises and caretakers passing by. Of course, commonly used behavioral data collection methods, such as scan sampling, 0-1 sampling, and on-site observation, also have their unique advantages. In terms of behavioral analysis, in addition to behavioral analysts, electronic data collection programs such as Noldus Observer and Mangold INTERACT are quite useful and we will try them in the future.

5 Conclusions

In this study, a behavior collection and analysis system for rhesus and cynomolgus monkeys was developed with the aim of providing a quantification analysis of the daily behaviors of rhesus and cynomolgus macaques, which are the most commonly used non-human primates in brain disease modeling studies. The system was developed based on an extensive literature review and our long experience conducting monkey behavioral research. It includes daily behavioral data collection protocols and a daily behavior ethogram (MDBE). As an application example, we then developed a sub-ethogram system based upon MCBE for evaluating the core clinical symptoms in an ASD monkey model by referring to the DSM-5-TR, a globally recognized authoritative tool for the classification and diagnosis of mental disorders.

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Data availability statement

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

This study was partially supported by Key-Area Research and Development Program of Guangdong Province (2019B030335001), STI2030-Major Projects (2021ZD0200900), National Key Research and Development Program of China (2021YFF0702700, 2018YFA0801403), National Natural Science Foundation of China (81960422, 32100801, 82101241, 82360226, 82160923, 82260929, 82374425, 32460194), Strategic Priority Research Program of the Chinese Academy of Sciences (CAS) (XDB32060200), STI2030-Major Projects (2022ZD0205200, 2022ZD0212700), Yunnan Province (202305AH340006, 202305AH340007), Yunnan Fundamental Research Projects (202201AT070153, 202201AT070139), Science and Technology Project of Yunnan Province (202101AY070001-001), Yunnan Revitalization Talent Support Program (YNWR-QNBJ-2019-043), CAS "Light of West China" Program, and "Yunnan Revitalization Talents Support Plan". We would like to thank the National Research Facility for Phenotypic and Genetic Analysis of Model Animals, Kunming Institute of Zoology, Chinese Academy of Sciences and National Resource Center for Non-Human Primates for technical support and animal care.

Author contributions

Xintian HU, Xiaoli FENG, Dongdong QIN, and Wenchao WANG designed and led the project. Xiaofeng REN, Huimin WANG, and Xiaoman LV conducted the literature research, combining the opinions of all authors to establish the two scales. Hao LI and Yingzhou HU gave constructive comments on the revision of the scales. Yingyin FAN, Yanjun YU, and Yuhui CHEN performed the collection and analysis of monkey behaviors. Yi ZHOU, Xiaofeng REN, Christoph W. TURCK, and Longbao LV performed statistical analysis of the behavioral results. Xiaofeng REN and Xintian HU prepared the manuscript. Wenchao WANG and Xiaoli FENG assisted with revision of the article. All authors read and approved the final version of the manuscript.

Compliance with ethics guidelines

Xiaofeng REN, Huimin WANG, Xiaoman LV, Yi ZHOU, Yingyin FAN, Yanjun YU, Christoph W. TURCK, Yuhui CHEN, Longbao LV, Yingzhou HU, Hao LI, Wenchao WANG, Dongdong QIN, Xiaoli FENG, and Xintian HU declare that they have no conflict of interest.

All animal welfare and experimental protocols were approved by Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences (No. IACUC-PE-2023-12-006). All monkeys were also handled in accordance with National Institute of Health (USA) Guide for Care and Use of Laboratory Animals. All animals had free access to clean water and were fed monkey chow supplemented with seasonal fresh fruit and vegetables twice a day. There were swings, ladders, branches and mirrors for enriching living habitats within each social cage. Visual and auditory stimuli were offered daily through television programs and music display for sensory enrichment of single-caged monkeys.

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Supplementary information

Table S1, S2, S3, S4; Fig. S1; Video clips

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