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Original Research

Videotaped Behavior as a Predictor of Clinical Outcome in Rhesus Macaques (*Macaca mulatta*)

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Understanding the behavior of laboratory NHP facilitates health assessment and clinical care. We sought to characterize the behavior of critically ill rhesus macaques (*Macaca mulatta*) and determine whether specific behaviors or behavioral changes might facilitate the determination of prognosis and clinical endpoints. Twenty-two critically-ill subjects were videorecorded after they were removed from the outdoor breeding colony for diagnostic work-up and treatment. Subjects were categorized as survivors (n = 15) and those that were euthanized according to existing clinical endpoints (n = 7). Behavior before, during, and after cageside examination was compared between these groups with regard to the presence or absence of direct observation. This approach allowed us to determine whether these settings revealed differences between groups or masking of behaviors during direct observation. Before cageside examination, several behaviors (for example, self-grooming and anxiety behaviors) were significantly more common in surviving subjects than in euthanized subjects. Few significant differences in behavior were detectable during or after the examination. Subjects that were eventually euthanized showed more illness-related behaviors; however, not all animals requiring euthanasia showed these signs when an observer was present. Furthermore, euthanized animals spent more time in an alert posture during direct observation than at other times. Therefore, direct observation of critically ill rhesus macaques may not yield the most accurate assessment of illness severity, and using video to assess behavior may be helpful for prognosis.

The assessment and recognition of pain and distress in laboratory animals is crucial to ensure welfare and high-quality research.[6,10,12,20,23,26,28] Difficulty in identifying species-specific signs of pain and distress suggests that cageside assessment of clinical condition by an observer, as a stand-alone method, may not be an optimal method of determining prognosis in critically ill animals of some species.[10,24,26,27] The behavior of NHP, including rhesus macaques (*Macaca mulatta*), can be especially difficult to interpret due to their relatively stoic nature and tendency to mask clinical signs of illness in the presence of human observers.[8,10,12,14,17] Two explanations for the tendency to mask pain, which is also observed in other species, are their status as a prey species and the need to hide weaknesses from group members.[3,17,22,23] According to the 2009 Institute for Laboratory Animal Research (ILAR) recommendations for recognizing pain and distress in nonhuman primates, "[v]iewing an animal from a distance or by video can aid in detecting subtle clinical changes."[12] The goal of the current study was to evaluate the use of videotaped behavioral data to increase accuracy in predicting prognoses for rhesus macaques that are critically ill.

A humane endpoint is defined as the earliest time at which an animal may experience unnecessary pain or distress, undue suffering, or impending death.[4,12] Both subjective and objective diagnostic criteria are currently used to define clinical and research endpoints.[6,7,10,12,21,26,29] A myriad of diagnostics can be used for this purpose, including physical examination, cageside examination, blood assays (CBC and serum biochemistry), imaging (radiographs, ultrasound, CT, and MRI), bacterial culture and sensitivity, urinalysis, and collection of tissue samples (fine-needle aspiration and biopsy). One specific example of using clinical diagnostics to aid in the prediction of mortality in NHP involved using acid-base levels in animals with severe social trauma.[11] Behavioral assessment may be an important and underutilized diagnostic method for assessing pain and distress. Recent studies have evaluated the behavior, specifically facial expression, of mice to detect and assess pain.[16,19] Methods for evaluating behavior typically involve cageside examination, but assessment of behavior via videotape in the absence of an observer may also be useful.

Endpoints may vary depending on species, nature of the research conducted, and assessment of degree of pain or distress. At our facility, experimental endpoints (those defined as part of a specific study) are differentiated from clinical endpoints (those determined by an animal's health and quality of life). The veterinarian has the authority to elect euthanasia for an animal that is assessed to have reached an endpoint by either or both classifications. IACUC-approved endpoint policies have been established to aid in this decision. Criteria included in these guidelines include weight loss (excluding postpartum females and intended weight loss for obese animals), anorexia that is not responsive to treatment, diarrhea that is not responsive to treatment, and major organ failure that is not responsive to treatment. Veterinarians use

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cageside examination in the overall assessment of animals and to aid in the decision to euthanize.

To our knowledge, the current study is the first to assess the behavior of critically ill rhesus macaques that controls for the cause of clinical presentation and to use observer presence or absence as an independent variable. To obtain information that may assist in refining clinical endpoints, we compared the behavior of critically ill rhesus macaques while an observer was absent, present, and recently present (labeled as preobservation, observation, and postobservation, respectively, in this text).

We hypothesized that in comparison to in-person observation of critically ill rhesus macaques, the use of videotaping in the absence of direct observation would be more accurate in detecting differences in the behavior between those animals that eventually required euthanasia and those that did not. In addition, we hypothesized that behaviors would be suppressed ('masked') during direct observation, accounting for the reduced information available to the direct observer. If our hypothesis is supported, then videotaped behavior may be useful as a prognostic indicator that could be incorporated into development of clinical endpoints.

Materials and Methods

Subjects and housing. All work was conducted at the Tulane National Regional Primate Center (Covington, LA), an AAALAC-accredited institution. Housing and care were provided in accordance with the standards set forth in the *Guide for the Care and Use of Laboratory Animals*[11] and the US Department of Agriculture's Animal Welfare Regulations.[1] All study procedures and methods were preapproved by the Tulane University IACUC. Subjects were selected from the Indian- or Chinese-origin rhesus macaques presenting to the breeding colony clinic from either the SPF or conventional colony. The SPF colony is antibody- and virus-negative for simian retrovirus and seronegative for simian T-lymphotrophic virus 1, SIV, and *Macacine herpesvirus 1.* On presentation to the clinic, subjects received a physical examination, CBC, serum biochemistry, and any additional diagnostics as deemed appropriate by the veterinarian. Animals were individually housed indoors in stainless steel cages sized in accordance with or in excess of primary cage space regulations. All cages were equipped with perches and multiple enrichment devices. Feeding enrichment—including fruit, vegetables, and foraging materials—was provided a minimum of 3 times each week. Macaques were maintained on a 12:12-h light:dark cycle with ambient temperature of 64 to 72 °F (17.8 to 22.2 °C) and 30% to 70% relative humidity. Subjects were fed a commercial diet formulated for nonhuman primates (Purina Diet 5037, PMI Feeds, St Louis, MO) twice daily, and water was provided ad libitum. Macaques that met clinical endpoints were anesthetized with tiletamine hydrochloride (8 mg/kg IM) and administered buprenorphine (0.01 mg/kg IM) prior to being given an overdose of pentobarbital sodium intravenously. Euthanasia was confirmed through cardiac auscultation. A designated board-certified veterinary pathologist performed the gross necropsy and histopathology to ensure consistent interpretation of the study findings.

Subjects were selected from animals that presented to the clinic for treatment between 1 December 2011 and 1 September 2012 and that met at least one of the following inclusion criteria: severe dehydration (more than 8% dehydrated) as assessed by the veterinarian; body condition score of 1/5 (hip bones easily palpable and likely visible; prominent facial bones, spinous processes, and ribs; minimal to no muscle mass palpable over ilium or ischium; anus possibly recessed between ischial callosities; body very angular; no subcutaneous fat layer to smooth out prominences);[7] clinical presentation consistent with *Shigella flexneri* enteric bacterial infection (diarrhea, hematochezia, with or without periodontal disease, and with or without clinical signs consistent with arthritis); and dystocia or suspected retained placenta.

A total of 22 rhesus macaques (15 female and 7 male) were enrolled in the study. Of the 22 cases, 7 ended in euthanasia because the animals reached clinical endpoints as defined according to institutional guidelines. The euthanized group consisted of 5 female macaques (age, 7 to 20 y) and 2 male macaques (age, 4 to 5 y). Three of the 7 animals in the euthanized group met more than one inclusion criteria for this study. Subjects for the survived group were drawn from a large population of animals that met inclusion criteria but were not euthanized. Animals in the survived group were selected for study on the basis of having a clinical presentation that was similar to those in the euthanized group; animals presenting with social trauma were not included in the survived group because no animals with this presentation were euthanized during the course of the study. Subjects in the survived group included 9 female macaques (age, 3 to 20 y) and 6 male macaques (age, 2 to 8 y). Thirteen of the 15 animals in the survived group met more than one inclusion criteria for this study.

Data collection. One study aim was to document behavior changes over the course of veterinary monitoring; therefore, all data were collected in association with in-person, cageside examinations because these are normally performed as a component of clinical assessment at our facility. One of 2 veterinarians, not involved in behavioral data collection or analysis, performed all cageside and hands-on examinations and made decisions regarding treatment and euthanasia. No study findings were used in making treatment decisions regarding study subjects, because data were coded after animals had been euthanized or returned to the breeding colony.

Videotaping began the day after presentation to the clinic, to ensure completion of initial treatment and to allow time for initial acclimation to the new environment. Videotaped data were collected on 3 separate days between the first and sixth day after presentation to the clinic. Animals were videotaped in 1.5-h sessions, beginning 1h prior to a 1400-h cageside examination and ending approximately 30 min after the examination. The video collected totaled 4.5-h (over 3 separate sessions) for each subject.

For each session, a tripod-mounted video camera was brought into the housing room, placed in front of the subject's cage, and removed once the session was completed. The first 15 min after camera set-up were not coded, to avoid short-term behavioral effects of disturbance caused by camera placement. The duration of the cageside examination varied between 2 and 5 min (approximately 3 min). During the cageside examination, the veterinarian stood in a location that did not block the video camera. The same person (AMG) coded all videotaped data by using Observer XT 10.0 software (Noldus Information Technology, Leesburg, VA). An exhaustive, mutually exclusive ethogram was used to document overall activity budgets as well as behaviors commonly interpreted to indicate pain and distress (Figure 1). All behaviors observed on video sessions were included in the ethogram.

Continuous focal sampling was used to quantify behaviors that typically occur in bouts, and behavior was collapsed for analysis

Clinical Signs of Illness • Hunched: ischial callosities on bottom of cage or on perch with knees bent toward chest, back rounded, and head dropped toward chest • Impaired locomotion: moving with forward intention but slowly with stiff gait • Lying down: chest or abdomen or dorsum and at least one leg in contact with ground • Dropping food: putting food in mouth then letting it fall out of mouth
Normal Stationary Position • Sitting down: ischial callosities on floor or perch; head is up and legs are in a passive position • Standing: plantar aspect of feet on ground/perch with stifles at a >45° angle or palmar aspect of hands in same plane as feet with body approximately parallel to floor
Normal Locomotion • Locomoting: moving with forward intention via legs and/or arms • Hanging from cage: hanging from top or sides of cage without moving toward a different area of the cage; majority of weight supported by the hand(s) • Brachiating: locomoting using only arms
Eating: chewing and swallowing food
Drinking: swallowing liquid (water or electrolyte drink mix)
Other Normal Activity: • Manipulating object: interacting with or showing interest in enrichment or objects (for example, locks) within or hanging on cage • Crouching and scanning room: palms of hands and feet on bottom cage with body lowered but not in contact with bottom of cage; concurrently moving eyes to observe room
Repetitive Abnormal Behavior (no other categories of abnormal behavior were observed) • Swinging from cage: using arm(s) while hanging from top of cage to propel body in a forward-and-backward or side-to-side movement at least 3 times • Stereotypic cage bar licking: using tongue to lick cage for more than 3 seconds with no indication that the area being licked has food and/or moisture present

Figure 1. Ethogram: continuous focal observations (converted to percentage of time observed).

into 6 categories: behaviors consistent with clinical signs of illness (including hunched position, impaired locomotion, lying down, and dropping food from mouth), normal stationary position, normal locomotion, eating, drinking, other normal activity, and repetitive abnormal behavior (Figure 1). Time spent performing these bout behaviors was converted to percentages of total time observed. Behaviors of short duration were quantified as all-occurrences point events and calculated as frequency per hour. These point event behaviors were collapsed into 4 categories for analysis: self-grooming, anxiety behavior, social behavior, and pressing hand to head (Figure 2).

Data analysis. Behaviors were analyzed in 3 conditions: preobservation (the 45 min prior to the entry of the observer for cageside examination; observation (the 2- to 5-min [mean, 3 min] cageside examination by a veterinarian in the room); and postobservation (the 15 min after the exit of the veterinarian from the room). The first 15 min after camera set-up were not coded, to avoid the effect of observer presence. For most categories of behavioral data analyzed, measures of skewness, kurtosis, and homogeneity of variance failed to meet required assumptions for parametric statistical tests; therefore, nonparametric statistical tests were conducted by using Statistica version 10 (Statsoft, Tulsa, OK). Mann–Whitney U tests were used to compare subjects that required euthanasia and those that survived. A 2-tailed α of 0.013 was selected for Mann–Whitney U tests after a Benjamini–Hochberg procedure to control for multiple comparisons was conducted.[2] For these tests, a P value of 0.026 was defined as the threshold for a trend. The significance of changes in the behavior of individual animals across the 3 conditions was determined by using Friedman tests with an α of 0.025 and a threshold of 0.05 for trends; significant results were followed by Wilcoxon matched-pairs tests.

Results

Pathology. Seven macaques were euthanized because they met established clinical endpoint criteria. Endpoints were achieved at a maximum of 38 d after the conclusion of the data collection. Gross necropsy findings for euthanized animals included amyloidosis, colitis, cardiomyopathy, and arthritis. Histology results for the 4 macaques that presented with clinical signs consistent with *Shigella flexneri* enteric bacterial infection included: amyloidosis (splenic, hepatic, nephric, mesenteric, lymph node, gastric, illeal, cecal, or uterine or multiple types), colitis, and severe chronic synovitis. Rectal bacterial cultures from these animals were positive for both *S. flexneri* and *C. coli* in 2 macaques but negative for the other 2. Histologic findings for the 2 macaques that presented with severe dehydration and poor body condition (but not consistent with *Shigella flexneri* enteric bacterial infection) included ulcerative jejunitis and peritonitis. Results of rectal bacterial culture revealed *Campylobacter coli* in one but were negative for the other macaque. Primary gross necropsy and histology findings in the animal that presented with suspect retained placenta were renal failure and left ventricular thrombosis.

Of the 15 macaques that survived, 6 met project criteria due to severe dehydration with or without poor body condition; 4 animals had *C. coli*, and 2 had negative rectal bacterial culture results. Of the 7 animals that had clinical signs consistent with *S. flexneri* enteric bacterial infection, 4 had both *S. flexneri* and *C. coli*, one had *S. flexneri* only, and 2 had culture results of *C. coli* only. The remaining 2 macaques that survived presented with dystocia or suspected retained placenta.

Survived group compared with euthanized group. Prior to observation, macaques that survived exhibited clinical signs of illness for less time than did those that were euthanized ($U = 9$, $P = 0.002$, Figure 3). This pattern remained consistent while the

Self-grooming
- Grooming: using hands, feet, or mouth to manipulate skin or pelage in a noninjurious manner
- Wound-picking: using hands, feet, or mouth to manipulate site of wound (perianal region in the case of diarrhea)

Anxiety Behavior
- Head or body shake: moving head and shoulders from right to left rapidly with lower body in a stationary position
- Scratching: using digits in repetitive back and forth motion at single location
- Yawning: opening mouth wide and exposing teeth; head often tossed back

Social Behavior:
- Lunging toward front of cage: moving at high speed toward animal or person
- Open mouth staring: displaying visual fixation with ears flattened against head, brow retracted, round mouth
- Barking: making hoarse, staccato low sounds
- Lip smacking: opening mouth repetitively and pressing lips together creating a soft noise
- Cage shaking: using limb(s) to vigorously move bars back and forth in an irregular motion
- Grunting: making short, repetitive, low guttural sounds; usually food-related
- Coo calling: making a clear-toned noise of medium pitch and intensity; usually food-related
- Screaming: making a shrill noise of high pitch and intensity
- Fear grimacing: displaying a grin-like facial expression with retraction of lips exposing clenched teeth; ears may be flat and body stiff

Pressing Hand to Head
- Using palmar aspect of hand to make firm contact with the forehead region of the face with no rubbing or scratching; brow often pushed rostrally toward periocular region

Figure 2. Ethogram: point samples (converted to frequency per hour).

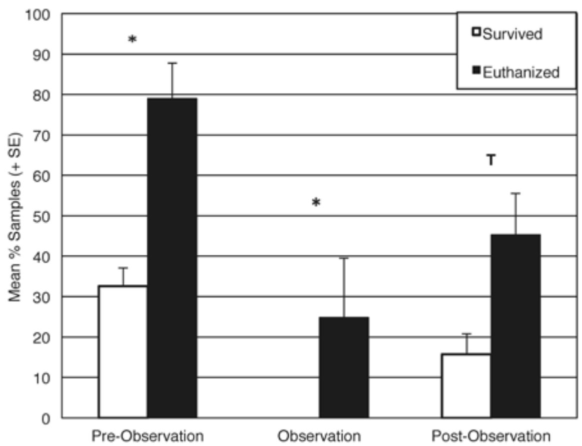

Figure 3. Clinical signs of illness prior to, during, and after cageside examination: survived group compared with euthanized group. *, $P < 0.013$; T, $P < 0.026$.

observer was in the room ($U = 15$, $P = 0.009$). There were no other differences in behavior between the 2 groups during direct observation. Immediately after cageside observation, macaques that survived tended to spend less time exhibiting clinical signs of illness ($U = 19$, $P = 0.02$) and more time exhibiting anxiety-related behaviors ($U = 19$; $P = 0.02$; survived, 12.05 ± 2.59; euthanized, 3.11 ± 1.14) compared with the euthanized group.

In addition to clinical signs of illness, macaques that survived exhibited several other behaviors before the observer entered the room at significantly greater proportions of time than did animals that were euthanized (Figure 4 A). Animals that survived spent greater proportions of time in normal stationary positions ($U = 13$, $P = 0.006$), performing normal locomotion ($U = 12$, $P = 0.004$), eating ($U = 15$, $P = 0.009$), drinking ($U = 11$, $P = 0.004$), and repetitive abnormal behavior ($U = 14$, $P = 0.007$) than did those that were euthanized. Surviving macaques also showed a trend toward higher levels of other normal activity ($U = 19$, $P = 0.02$). Macaques that survived displayed higher frequencies of self-grooming ($U = 6$, $P = 0.001$) and behaviors consistent with anxiety ($U = 11$; $P = 0.004$) as well as a trend toward a higher frequency of social behavior ($U = 19$, $P = 0.02$; Figure 4 B).

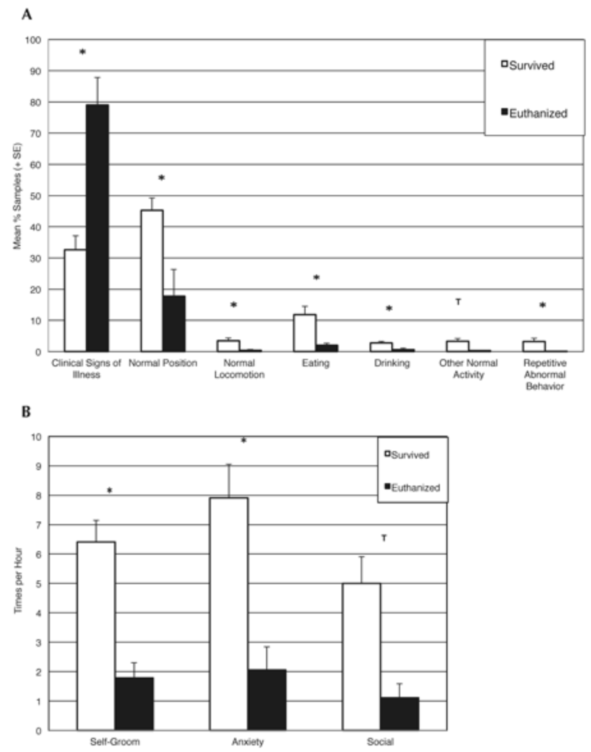

Figure 4. Behavioral contrasts during preobservation phase. (A) Bout behaviors. (B) Frequency behaviors. *, $P < 0.013$; T, $P < 0.026$.

Comparison of behaviors across phases: survived group. Friedman tests detected significant differences across observation phases for the following behaviors: clinical signs of illness (χ^2 [2, $n = 15$] = 24.67, $P < 0.0001$), drinking (χ^2 [2, $n = 15$] = 17.05, $P < 0.0002$), repetitive abnormal behavior (χ^2 [2, $n = 14$] = 10.58, $P < 0.005$), self-grooming (χ^2 [2, $n = 15$] = 21.80, $P < 0.0001$), anxiety behavior (χ^2 [2, n = 15] = 20.83, $P < 0.0001$), and pressing hand to head (χ^2 [2, n = 15] = 9.87, $P < 0.007$). Prior to cageside veterinary observation, animals that survived spent a greater proportion of time exhibiting clinical signs of illness (z = 3.41, P = 0.001), drinking (z = 3.41, P = 0.0007), and performing repetitive abnormal behaviors (z = 2.28; P = 0.023) compared with the period during which an observer was in the room (Figure 5 A). Prior to cageside observation, there was also a higher frequency of self-grooming (z = 3.41, P = 0.007), anxiety (z = 3.41, P = 0.007), and pressing hand to head (z = 2.37; P = 0.018) compared with during observation (Figure 5 B). Compared with levels during the preobservation period, subjects showed lower levels of clinical signs of illness during the postobservation period (z = 2.84, P = 0.005, Figure 5 A).

Comparison between observation and postobservation showed that while an observer was in the room, macaques that survived spent less time exhibiting clinical signs of illness (z = 3.06, P = 0.002), drinking (z = 2.8, P = 0.005), performing repetitive abnormal behaviors (z = 2.67, P = 0.008), self-grooming (z = 3.3, P = 0.001), anxiety-related behavior (z = 3.18, P = 0.001) and pressing hand to head (z = 3.1, P = 0.002).

Comparison of behaviors across phases: euthanized group. Friedman tests detected significant differences across observation phases for clinical signs of illness (χ^2 [2, n = 7] = 12.29, $P < 0.002$) and trends toward differences in normal posture (χ^2 [2, n = 7] = 6.89, $P < 0.03$) and anxiety behavior (χ^2 [2, n = 15] = 6.91, $P < 0.03$; Figure 6). Macaques that were euthanized spent more time engaged in behaviors associated with clinical signs of illness prior to cageside observation than during either of the subsequent phases (observation: z = 2.37, P = 0.02; postobservation: z = 2.37, P = 0.02) and showed a trend toward less time in a normal position than during the other phases (observation: z = 2.20, P = 0.03; postobservation: z = 2.20, P = 0.03; Figure 6). Last, euthanized animals tended to spend more time engaged in anxiety-related behavior after observation (3.11 ± 1.14; z = 2.20; P = 0.03) than prior to observation (2.06 ± 0.78).

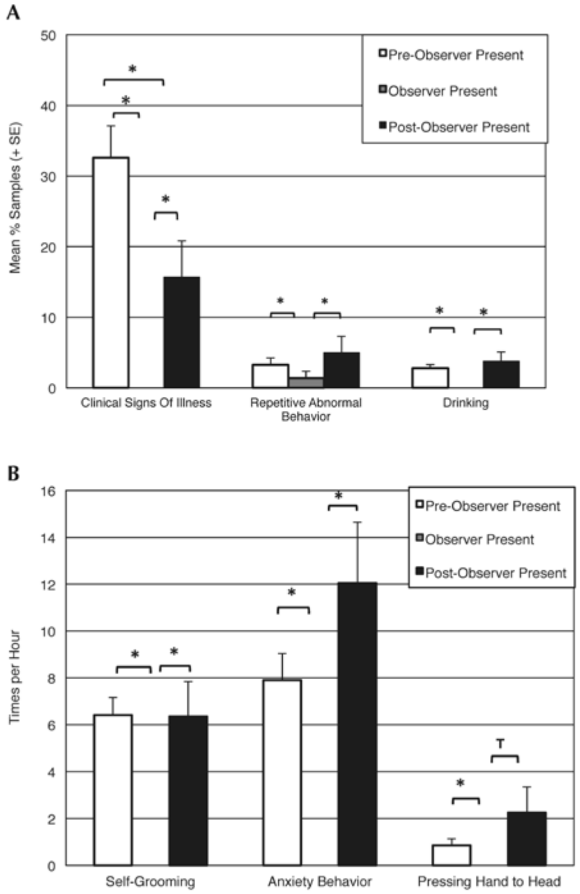

Figure 5. Effects of observer phases in the survived group. (A) Bout behaviors. (B) Frequency behaviors. *, $P < 0.025$; T, $P < 0.05$.

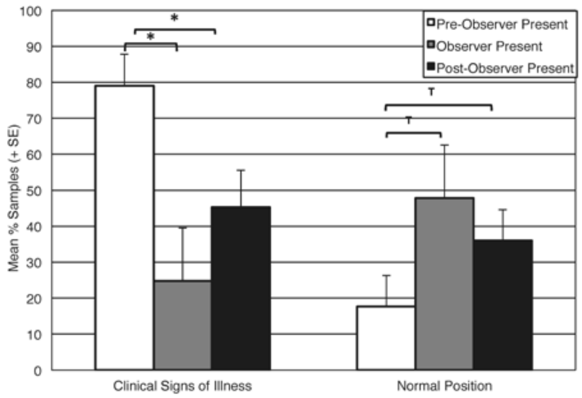

Figure 6. Effects of observer phases in the euthanized group: frequency behaviors. *, $P < 0.025$; T, $P < 0.05$.

Discussion

Minimization of pain and distress and the establishment of the most appropriate clinical endpoints are important goals in the field of laboratory animal medicine. Continued refinement of diagnostics is one method of ensuring that this goal is achieved. Compared with the process in humans, it is challenging to assess the degree of pain in animals. Prey species (such as NHP), which can hide signs of pain and distress, are particularly challenging in this regard.[3,17,22,23]Another diagnostic challenge is the rapidity with which severe disease states can progress. Determining the most accurate endpoint can be difficult when an animal's health rapidly declines.

The results of the current study indicate that regardless of clinical outcome, animals spend less time exhibiting signs of illness when an observer is in the room performing a cageside examination than when no observer is present. In all 3 of the observation phases, macaques that ultimately were euthanized spent a greater proportion of time exhibiting clinical signs of illness (hunched with head down, impaired locomotion, lying down, and dropping food from mouth) than did those that survived. Prior to veterinary observation, animals that were euthanized spent a mean of 79% of time exhibiting clinical signs of illness, whereas those that survived spent a mean of 33% of time exhibiting such signs. During cageside observation, animals that survived showed no clinical signs of illness, but those that were euthanized displayed clinical signs of illness 25% of time on average. During postobservation, macaques in both the survival and euthanasia groups tended to spend less time exhibiting clinical signs of illness (16% and 45%, respectively) compared with preobservation. This difference is likely to due to the lingering effects of direct observer presence on behavior. The finding that only macaques that were euthanized exhibited clinical signs of illness when an observer was present emphasizes the value of providing immediate cageside veterinary examination when a rhesus macaque appears clinically ill. This finding underscores the important diagnostic value of cageside examination, given that the prognosis is poor for macaques that show these behaviors in this setting. However, not all animals that were euthanized exhibited clinical signs of illness during the cageside examination. Furthermore, whereas all masking in the surviving animals involved the suppression of behaviors, macaques that required euthanasia not only failed to show this pattern for most behaviors, but in fact spent more time in a normal, alert posture when the observer was in the room than either before or after their cageside examination. Therefore, in conjunction with cageside examination, the assessment of behavior by videotape may be a valuable tool for determining the degree to which behaviors associated with illness are being masked by an animal. It appears that the demonstration during direct observation of behaviors that are normally masked is indicative of a poor prognosis.

Macaques that survived displayed behaviors associated with anxiety for a greater proportion of time when an observer was not present or had recently been present than they did before observation. In addition, animals that survived spent a longer proportion of time exhibiting repetitive abnormal behaviors when no observer was present in the room (postobservation > preobservation > observation). A prior study similarly found that abnormal behavior increases after an observer is present.[18] The increase in anxiety-related behaviors postobservation is consistent with that finding and therefore may be associated with recent direct observation. Although anxiety-related and repetitive abnormal behaviors are potential signs of impaired psychologic wellbeing, they may indicate a better prognosis for survival in the types of clinical cases involved in the current study. Rhesus macaques that were euthanized tended to spend less time performing normal activities such as object manipulation and scanning of the room, and perhaps spend more time in passive positions to conserve energy.

To our knowledge, pressing hand to head has not been previously described or analyzed in the literature. Additional discussion with staff at this facility revealed that this behavior is seen very occasionally on video but has never been observed in person. The cause of this behavior is unknown. In this study, hand-to-head behavior occurred only when an observer was not present and when animals were sitting passively. The behavior was manifested by placing the palmar aspect of the hand to the forehead and pressing into the brow (often pressing the brow rostrally). Animals that were euthanized rarely exhibited this behavior (and only during preobservation). This behavior may be associated with pain (that is, a pain-reduction mechanism through manual pressure), an abnormal behavior, or a displacement behavior (an anxiety-related behavior that can occur with social tension, although this behavior has never been noted in studies of anxiety-related behavior).[29] Because this behavior was observed at a higher frequency in animals that survived than were euthanized, hand-to-head behavior may be associated with anxiety, which was also higher in animals that survived. However, additional investigation regarding the underlying cause of this behavior is necessary for correct interpretation. Given the frequency of this behavior and the consistency with which it occurred preobservation in animals that survived, it may be prognostic for positive clinical outcome in rhesus macaques.

Although behavioral differences were compared and analyzed according to outcome and the presence (or lack thereof) of an observer, behavior also can vary with the type of clinical presentation, social rearing history, sex, hormonal influence, position within the social hierarchy, and housing location changes within the clinic.[5,9,15,17,23,25] An important aspect of the study design was to restrict test cases to subjects that eventually required euthanasia and to those with similar clinical presentations that survived. Potential subjects presenting with other common clinical problems from which all of these animals survived (for example, social trauma) were excluded from the study by design. Results of the current study may not apply to all clinical presentations, suggesting a direction for future study. Nonetheless, our study controlled for the potential confounding effect of the type of clinical presentation. Standardizing the elapsed time between the date of arrival in the clinic and the onset of data collection controlled for the varied housing. Despite possible confounds, our study found several significant effects, thereby affirming that videotaping can assist in the determination of prognosis for critically ill rhesus macaques.

Data for behavioral analysis through direct observation should be collected once an observer is able to approach the subject with minimal behavioral reaction.[14] However, animals in critical condition must be assessed immediately for clinical signs of pain or distress. Therefore, an extended period of acclimation to direct observation is impractical for obtaining behavioral information in these cases. Videotaping allows behavioral assessment without an observer effect. As the current research demonstrates, critically ill rhesus macaques mask their pain and discomfort in the presence of an observer and in the period immediately after an observer leaves the room. Animals with a poor prognosis may not express clinical signs of illness during cageside examination. Most subjects that were euthanized did not exhibit significant clinical signs of illness on cageside examination despite showing them prior to the examination. A standardized direct cageside observation for a longer duration could be considered for future study. This design could provide more information about how long rhesus macaques will mask clinical signs of illness and other behavioral changes after an extended exposure to a human observer. One would have to consider the value of adding more coding time to case assessment, especially when several critical cases are assessed concurrently.

Although the time required to set up the video camera was minimal and could be incorporated into the duty of any staff member, the use of an automated video camera mounted to the wall in a room by using either remote access or recording on a timer could permit more efficient monitoring by videotape. More importantly, an automated system may be beneficial for observing behavior without the changes associated with recent observer presence. The use of this technology would be especially beneficial in quarantine, ABSL3, and ABSL4 facilities, where direct access to animals is complicated or limited.

The current study assessed the use of video as an additional diagnostic tool to enhance ability to develop accurate prognoses and to predict outcomes for animals in critical condition. Rhesus macaques exhibited distinct differences in behavior depending both on whether a human observer was in the animal housing room and whether the animals ultimately survived or were euthanized. Our findings support our hypothesis that less information is available to a clinician in person than can be gleaned from video. Clinical signs of illness in rhesus macaques are suppressed during direct observation and may not be shown by all animals that will require euthanasia. However, numerous other behaviors on video may predict outcome, particularly those that are seen prior to the cageside examination. These differences are likely to be missed during in-person cageside examination. Videorecording may facilitate an accurate and detailed clinical assessment and help to refine clinical endpoint criteria for rhesus macaques.

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References

1. **Animal Welfare Regulations.** 2008. 9 CFR § 3.129.
2. **Benjamini Y, Hochberg Y.** 1995. Controlling the false-discovery rate: a practical and powerful approach to multiple testing. J Royal Statist Soc B (Methodological) **57:**289–300.
3. **Bohm RP, Gilbert MH.** 2012. Emergency medicine and critical care for nonhuman primates, p 359–389. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. Nonhuman primates in biomedical research: biology and management, vol 1. Waltham (MA)): Elsevier.
4. **Canadian Council on Animal Care.** 1998. Guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching, and testing.
5. **Capitanio JP.** 2011. Nonhuman primate personality and immunity: mechanisms of health and disease, p 233–255. In: Weiss A, King JE, Murray L, editors. Personality and temperament in nonhuman primates, vol 1. New York (NY): Springer.
6. **Carstens E, Moberg GP.** 2000. Recognizing pain and distress in laboratory animals. ILAR J **41:**62–71.
7. **Clingerman KJ, Summers L.** 2005. Development of a body condition scoring system for nonhuman primates using *Macaca mulatta* as a model. Lab Anim (NY) **34:**31–36.
8. **Coleman K, Bloomsmith MA, Crockett CM, Weed JL, Schapiro SJ.** 2012. Behavioral management, enrichment, and psychological wellbeing of laboratory nonhuman primates, p 149–176. In: Abee CR, Mansfield K, Tardif S, Morris T, editors. Nonhuman Primates in biomedical research: biology and management, vol 1. Waltham (MA): Elsevier.
9. **Fillingim RB, Ness TJ.** 2000. Sex-related hormonal influences on pain and analgesic responses. Neurosci Biobehav Rev **24:**485–501.
10. **Hawkins P.** 2002. Recognizing and assessing pain, suffering, and distress in laboratory animals: a survey of current practice in the UK with recommendations. Lab Anim **36:**378–395.
11. **Hobbs TR, O'Malley JP, Khouangsathiene S, Dubay CJ.** 2010. Comparison of lactate, base excess, bicarbonate, and pH as predictors of mortality after severe trauma in rhesus macaques (*Macaca mulatta*). Comp Med **60:**233–239.
12. **Institute for Laboratory Animal Research.** 2009. Recognition and alleviation of pain in laboratory animals. Washington (DC): National Academies Press.
13. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
14. **Iredale SK, Nevill CH, Lutz CK.** 2010. The influence of observer presence on baboon (*Papio* spp.) and rhesus macaque (*Macaca mulatta*) behavior. Appl Anim Behav Sci **122:**53–57.
15. **Jovanovic T, Maestripieri D.** 2010. Effects of early traumatic experience on vocal expression of emotion in young female rhesus macaques. Dev Psychobiol **52:**794–801.
16. **Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Click S, Ingrao J, Klassen-Ross T, LaCroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maggdenberg AMJM, Ferrari MD, Craig KD, Mogil JS.** 2010. Coding of facial expressions of pain in the laboratory mouse. Nat Methods **7:**447–449.
17. **Lefebvre L, Carli G.** 1985. Parturition in nonhuman primates: pain and auditory concealment. Pain **21:**315–327.
18. **Line SW.** 1995. Effects of observation techniques on the behavior of adult rhesus macaques. Contemp Top Lab Anim Sci **34:**61–65.
19. **Matsumiya LC, Sorge RE, Sotocinal SG, Tabaka JM, Wieskopf JS, Zaloum A, King OD, Mogil JS.** 2012. Using the mouse grimace scale to reevaluate the efficacy of postoperative analgesics in laboratory mice. J Am Assoc Lab Anim Sci **51:**42–49.
20. **Mayer J.** 2007. Use of behavior analysis to recognize pain in small mammals. Lab Anim (NY) **36:**43–48.
21. **McCowan B, Beisner BA, Capitanio JP, Jackson ME, Cameron AN, Seil S, Atwil ER, Fushing H.** 2011. Network stability is a balancing act of personality, power, and conflict dynamics in rhesus macaque societies. PLoS One **6:**e22350.
22. **National Research Council.** 1998. The psychological wellbeing of nonhuman primates. Washington (DC): National Academies Press.
23. **Plesker R, Mayer V.** 2008. Nonhuman primates mask signs of pain. Lab Prim Newsletter **47:**1–3.
24. **Rommeck I, Capitanio JP, Strand SC, McCowan B.** 2011. Early social experience affects behavioral and physiological responsiveness to stressful conditions in infant rhesus macaques (*Macaca mulatta*). Am J Primatol **73:**692–701.
25. **Smith JJ, Hadzic V, Li X, Liu P, Day T, Utter A, Kim B, Washington IM, Basso MA.** 2006. Objective measures of health and wellbeing in laboratory rhesus monkeys (*Macaca mulatta*). J Med Primatol **35:**388–396.
26. **Stasiak KL, Maul D, French E, Hellyer PW, Vandewoude S.** 2003. Species-specific assessment of pain in laboratory animals. Contemp Top Lab Anim Sci **42:**13–20.
27. **Stokes WS.** 2002. Humane endpoints for laboratory animals used in regulatory testing. ILAR J **43:**S31–S38.
28. **Trammell RA, Toth LA.** 2011. Markers for predicting death as an outcome for mice used in infectious disease research. Comp Med **61:**492–498.
29. **Troisi A.** 2002. Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects. Stress **5:**47–54.