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Changes in spontaneous activity assessed by accelerometry correlate with extent of cerebral ischemia-reperfusion injury in the nonhuman primate

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Abstract

The use of accelerometry to monitor activity in human stroke patients has revealed strong correlations between objective activity measurements and subjective neurological findings. The goal of our study was to assess the applicability of accelerometry-based measurements in experimental animals undergoing surgically-induced cerebral ischemia. Using a nonhuman primate cortical stroke model, we demonstrate for the first time that monitoring locomotor activity prior to and following cerebrovascular ischemic injury using an accelerometer is feasible in adult male rhesus macaques and that the measured activity outcomes significantly correlate with severity of brain injury. The use of accelerometry as an unobtrusive, objective preclinical efficacy determinant could complement standard practices involving subjective neurological scoring and magnetic resonance imaging in nonhuman primates. Similar activity monitoring devices to those employed in this study are currently in use in human clinical studies, underscoring the feasibility of this approach for assessing the clinical potential of novel treatments for cerebral ischemia.

Keywords

accelerometer; actigraphy; ischemia; nonhuman primate; rhesus macaque; stroke

Introduction

Impaired physical activity following stroke has a profound negative impact on the quality of life of stroke patients and their families. Approximately 700,000 people suffer from stroke

each year in the United States. Of the 4.8 million Americans currently diagnosed with stroke, over one million are reported to have ongoing problems carrying out activities related to daily living [1]. Stroke often results in a substantial impairment in motor activity with the degree of disability following stroke highly dependent upon the area and extent of the brain that is affected. Quantification of the extent of motor deficits following stroke can therefore be problematic and has been limited historically to the use of subjective neurological scales [2]. While these sophisticated neurological scoring systems have great value, obvious limitations are the discontinuous view provided of the status of motor paresis of the subject and significant expertise required to implement. Additionally, neurological scales differ in their emphases on specific deficits and often are insensitive to subtle changes in motor function [2]. In a preclinical setting, establishing sensitive, unbiased, and quantitative measures of drug efficacy is essential to successful drug development.

In the search for reliable and objective measurements of motor activity, lightweight miniaturized accelerometer devices offer great promise. The major advantage of accelerometer activity monitoring is that it enables the continuous objective evaluation of motor activity using a non-invasive, safe, and convenient method. Data derived from miniaturized accelerometers have recently been validated for use as objective measures of total physical activity [3] and to evaluate the extent of motor impairment and quantitatively assess post-stroke rehabilitation and recovery outcomes in humans [4]. We postulate that these devices can be used in preclinical development to quantitatively assess neurological outcomes in nonhuman primate (NHP) models of stroke.

Nonhuman primates are useful for modeling human pathologies because they exhibit a range of activities quite similar to humans. A novel NHP model of stroke using a two-vessel occlusion model was developed recently by our laboratory [5]. In this model, quantitative information about the degree of brain infarction and location of injury is obtained by magnetic resonance imaging (MRI) [5]. In addition, motor function is assessed subjectively using complex neurological scales reflective of human stroke scales [6]. Although functional outcomes are a desired measure for preclinical studies, subjective measurements of neurological deficits are difficult to apply to NHP due to an inability to accurately detect cognitive or motor impairments. Furthermore, implementation of neurological scoring systems in NHP studies is labor intensive and requires sufficient knowledge of typical NHP behavior, as well as clinical neurology. In contrast, application of accelerometer-based activity monitoring to NHP stroke models, whereby one monitors activity in a non-invasive and unbiased fashion, could provide a more practical, continuous and reproducible measure of the neurological deficits resulting from stroke.

Although application of this technology to experimental animal stroke models has not previously been reported, activity monitoring has been applied to behavior and physiology studies in the rhesus macaque by attaching accelerometers to lightweight neck collars [7-10]. Studies by Papailiou *et al.* [8] support the hypothesis that collar-mounted activity monitors allow effective quantification of activity in rhesus macaques. These studies showed that omnidirectional accelerometers attached to collars measure whole body movements (i.e., movement of body over distance, continuous repetitive movement, jumping), whereas other behaviors (i.e., chewing, grooming, toy manipulation, and arm movement) do not significantly influence overall activity levels. These data suggested that recording whole body movement using collar worn accelerometers would provide a continuous quantitative measure of locomotor activity and may be a valuable parameter for NHP stroke models. More importantly, activity monitoring could be of significant value as a more objective measure for the evaluation of novel clinical therapeutics, and could provide a means to more effectively compare results from multiple studies from different laboratories.

The purpose of this study was to determine whether the monitoring of physical activity over 24-hour activity-rest cycles by means of an Actiwatch recording device could be implemented as an additional objective measure to quantify changes in spontaneous motor activity in a rhesus macaque stroke model. Importantly, in this NHP model of cerebral ischemic injury we sought to determine whether 1) quantitative results from continuous activity monitoring correlate with the extent of brain damage measured by MR imaging, 2) activity results correlate with subjective neurological scoring parameters and 3) differences in the temporal distribution of activity over a 24-hour activity-rest cycle could be discerned. The importance of this work is that by using these methods it may be possible to simplify neurological assessments in an experimental stroke model and simultaneously improve the quality of motor function data using a non-invasive approach with minimal bias.

MATERIALS AND METHODS

Animal husbandry

Adult male Chinese rhesus macaques (*Macaca mulatta*, n=23) were cared for by the Division of Animal Resources at the Oregon National Primate Research Center (ONPRC) in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The animal care program is compliant with federal and local regulations regarding the care and use of research animals and is Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC). Animals were housed indoors under controlled conditions at a constant temperature of $24 \pm 2^{\circ}\text{C}$, 12L:12D photoperiods. Room lights (~300 lux) were set to a 12-hour light/dark cycle (0700 h to 1900 h) daily. Animals were subject to morning observations (0730 h to 0800 h), and enrichments (1300 h to 1400 h). Animals received regular meals at 0830 h and 1500 h (Purina High Protein Monkey Chow, Purina Mills, Inc., St. Louis, MO) supplemented with juice, fresh fruit, vegetables and candy treats; fresh drinking water was available *ad libitum* (**Fig. 1**) during acclimation period. Animals received a variety pan of food post-stroke to stimulate appetite and were given food treats during neurological assessments. Adult male rhesus macaques between six and twelve years of age were housed individually in a double cage within a single room. In addition, room washing was performed daily between 1000 h and 1100 h, except for the days following surgically-induced stroke.

Animal selection and acclimation

Approximately 3 weeks prior to stroke surgery, adult male rhesus macaques were examined for general health by the attending veterinarian and then moved to their study cages for acclimation. At this time an Actiwatch activity monitor was attached to each animal's collar and a blood sample was collected to help identify potential health concerns. Animals were selected based on normal results from a physical examination and/or normal clinical laboratory findings. Exclusion criteria included: 1) abnormal hematology and/or clinical chemistry values outside of 2SD from mean of our historical ranges in adult male Chinese rhesus macaques (data from >60 individual age/sex-matched controls), 2) recent invasive procedures (e.g., dental cleaning or tooth extraction), 3) presence of serum cytokines or other indicators of inflammation or infection (e.g., tooth abscess, high c-reactive protein levels, visible injury, chronic diarrhea), 4) neurological disorders evident by abnormal motor or cognitive abilities, or 5) stress-associated behaviors.

Surgical protocol

Cerebral ischemia was induced using procedures previously described [11] for the purpose of testing the efficacy of novel therapies for stroke. Briefly, up to four weeks before surgery, animals were screened for general health, endemic disease, and neurological disorders.

Animals were given ketamine (~10 mg/kg, intramuscular injection) and then intubated and maintained under general anesthesia using 0.8% to 1.3% isoflurane vaporized in 100% oxygen. A blood sample was taken and a venous line was placed for fluid replacement. An arterial line was established for blood pressure monitoring throughout surgery and to maintain a mean arterial blood pressure of 60-80 mm Hg. End-tidal CO₂ and arterial blood gases were continuously monitored to titrate ventilation to achieve a goal PaCO₂ of 35-40 mm Hg. The right middle cerebral artery (distal to the orbitofrontal branch) and both anterior cerebral arteries were exposed and occluded with vascular clips for 60 or 75 minutes, as previously described [11, 5]. Surgical procedures were conducted by a single surgeon. Post-operative analgesia was given on Days 1 and 2 post-op consisting of intramuscular hydromorphone HCl and buprenorphine. Animals were monitored closely for the 7 study days following stroke.

Infarct volume measurements

Infarct volume was measured from T2-weighted MRI performed after 2 days of reperfusion as described previously [5]. All scans were performed on a Siemen's 3T Trio system, housed on-campus near the surgical suite at ONPRC. Because of the small filling capacity of the rhesus macaque head, a human extremity coil was used to achieve better image quality of the brain. Animals were given ketamine (10 mg/kg intramuscularly) and a blood sample collected. Animals were then intubated and administered 1% isoflurane vaporized in 100% oxygen for anesthesia maintenance. All animals received numerous anatomical MRI scans. The T2 scan was a turbo spin-echo protocol, with TR=5280 ms, TE=57 ms, number of averages=4, an echo train length of 5, and a refocusing pulse flip angle of 120°. The entire brain was imaged with a 0.5 × 0.5 mm in-plane resolution and a slice thickness of 1 mm. Images from T2-weighted MRIs were examined for the location of infarction, and the total affected area measured using ImageJ, as previously described [5] by sampling approximately 15 slices (4 mm each). Measurements of infarct volume as a percentage of the ipsilateral hemisphere were made using the following formula: (volume of infarcted tissue of the ipsilateral hemisphere / total volume of the ipsilateral hemisphere) x100%.

Neurological assessments in NHP

Neurological assessments were performed between 0700 and 0830 by a single observer as previously described for this model [11] using a scoring system adapted from that previously described [6]. This strategy was chosen to ensure neurological evaluations were performed at the same time each day prior to the administration of analgesics, when given. Our scale evaluates motor function and behavior (mental status) with higher scores representing better functional outcomes (100=normal). Motor function is scored daily from 1 to 70, according to severity of hemiparesis in the left extremities. A score of 10=severe hemiparesis, 25=moderate hemiparesis, 40= slight hemiparesis, 55=favors normal side, or 70=normal ability. Behavior and alertness are scored daily ranging from 1 to 20, with 1=unresponsive, 5=aware but inactive, 15=aware but less active, and 20=normal. Facial deficit was scored as 1=one-sided paralysis or 5=normal facial movement. Visual deficit was scored as 1=present and 5=absent. As administration of analgesics were found to have a minimal effect on our provoked assessments, values reported are cumulative scores reflecting the sum of the scores from days 1-7 post-stroke (700 maximum possible score).

Actiwatch device

The Actiwatch activity monitor consists of a piezoelectric accelerometer with 64 kilobytes of memory capacity [10], which records the integration of intensity, amount, and duration of movement in all directions with a force sensitivity of 0.05 g and a maximum sampling frequency of 32 Hz. Each animal was fitted with an Actiwatch (Philips-Respironics, Bend, OR, USA; part number U198-0301-00) placed inside a protective case (Philips-Respironics;

part number 198-0232-00 M) and then attached to a lightweight loose-fitting aluminum collar (Primate Products, Inc., Immokalee, FL) approximately 3-4 weeks prior to surgery, as described previously [9]. Continuous recordings were made approximately 21 days prior to surgery and up to 8 days after surgery. Devices were programmed to collect data in 60-second epochs and data were downloaded using a dedicated reader (Philips-Respironics; part number 198-0150-00). Data were interpreted and actograms drawn using Actiware-Sleep version 3.4 software (Cambridge Neurotechnology Ltd, Cambridge, United Kingdom). The mean daytime activity (defined as activity during the period between 0700 h and 1859 h) and mean nocturnal activity (activity between 1900 h and 0659 h) were calculated. In addition, the mean total daily activity defined as the sum of the daytime and nocturnal activity, as well as the ratio of day to nocturnal activity defined as the daytime activity divided by the nocturnal activity, were tabulated prior to stroke and after stroke. Individual daily activities from the 4 consecutive days the week prior to surgery (**Fig. 1**) were tabulated generating a mean baseline value according to the following: mean baseline activity = $(A_{\text{Day-7}} + A_{\text{Day-6}} + A_{\text{Day-5}} + A_{\text{Day-4}}) / 4$; where A=activity. We found that the administered analgesics affected activity measures on days 1 and 2 post-stroke over the 24-hour period. Therefore, activities of Days 3 thru 6 post-surgery (comprised of days following the cessation of post-surgical drug administration) were used to determine post-stroke changes in activity. The percent change from mean baseline value was calculated for each of the four days using the following equation: % change = $(\text{post-stroke activity (Day X)} / \text{mean baseline activity}) * 100$. The mean % change post-stroke was then calculated. Animals sacrificed prior to Days 3-6 post-stroke were assigned a value of 100% as a mean reduction in activity.

Data exclusions

Data show that rhesus macaques typically demonstrate stable daily circadian rhythms of motor activity [12]. As an *a priori* exclusion criterion for this analysis, we retrospectively evaluated this parameter for each animal in our study. Day-to-day changes in total activity were determined for each animal by examining Days -7 to -4 from the week prior to surgery for individual animals according to the following equation: mean % change in day-to-day activity = $(\% \text{ change in activity Day-7 to -6} + \% \text{ change in activity Day -6 to -5} + \% \text{ change in activity Day -5 to -4}) / 3$. The mean ($\pm$ SEM) change in day-to-day activity was $14\% \pm 3\%$ for the entire cohort (n=21). A single animal demonstrated 53% mean change in activity level day-to-day and as such was excluded from all analyses as a significant outlier for baseline activity ($>2.5\text{SD}$ from the mean of the group). The reason for unstable baseline activity levels in this animal was unclear.

Data and statistical analyses

Data described in the text reflect mean $\pm$ SEM unless otherwise noted. All statistical analyses were performed using Prism 5.0 software (GraphPad Software, La Jolla, CA). Correlations between parameters were determined using Pearson's correlation. Differences were considered statistically significant when two-tailed $p < 0.05$. It was necessary to terminate five animals with large infarcts at two days following surgical occlusion. For analysis of neurological data, the last measured observation was carried forward (LOCF) for subsequent days when animals were terminated prior to the end of the study. The LOCF method is often used for clinical data to handle attrition or incomplete longitudinal data. In addition, our historical data show that little if any improvement occurs in severely affected animals beyond day 3. The LOCF method in our case offers less bias as the final measured carried forward likely underestimates rather than overestimates the severity of neurological deficits for days following termination. For data analysis of activity data, animals were assigned a value of 100% decrease in activity. The LOCF method could not be used in this case since activity measurements taken at day 2 prior to termination were compromised by the administration of post-stroke analgesics. We justify our analysis approach since animals

with severe infarcts (>25% of hemisphere) surviving to the end of the study had >80% reduction in activity levels for all but nocturnal activity. In addition, activity levels, like neurological scores, do not typically show significant improvement over this 7-day time period in this stroke model. Nevertheless, statistical analyses of these data were performed with both the inclusion and the exclusion of these early terminated animals. We found that the statistical significance was independent of data from these animals ($p < 0.05$ for all correlations), with the exception of nocturnal activity ($p = 0.97$ versus infarct and $p = 0.32$ versus neurological score), which did not achieve statistical significance without the inclusion of data from animals terminated prior to study end at day 7 post-stroke.

RESULTS

Locomotor Activity Levels Change Following Cerebral Ischemic Injury

Following cerebral ischemic injury induced by two-vessel surgical occlusion, adult male rhesus macaques demonstrated a range of phenotypes spanning from mild hemiparesis (reduced movement) to paralysis (no movement) of upper and/or lower limbs. Facial paresis was also commonly observed, as demonstrated by an asymmetrical grimace and difficulty chewing on the affected side. Distal plegia of the upper limb (hand) was more commonly present while lower limb deficits were seen rarely and varied in severity, although when present, mild paresis of distal lower limb was most commonly observed. Behavioral changes were varied but the majority of affected animals generally were alert and responsive with good mentation, although decreased aggression and fear responses were sometimes observed. Following experimental stroke, the animals demonstrated reduced activity levels, a phenotype that has not previously been quantified in NHP stroke models and our study specifically aimed to quantify these changes.

Changes in Activity Levels Correlate with the Extent of Brain Injury

The extent of cerebral ischemic injury following surgical occlusion can be measured using an MRI approach that derives infarct volumes as a percent of hemisphere. Previous studies show that infarct volume determined by MRI at day 2 post-stroke correlate with histological findings [5]. Infarct volumes in this study ranged from 0 to 37% of ipsilateral hemisphere at 2 days following occlusion. We hypothesized that the resulting infarct volume would be proportional to the change in activity level, in that animals with larger infarcts would demonstrate greater decreases in activity after stroke as compared to their baseline values. As predicted, the percent change in post-stroke total daily activity (**Fig. 2A**) and daytime activity (**Fig. 2B**) compared to baseline values significantly correlated with infarct volume. These data reinforce the notion that a dramatic decrease (~80%) in the magnitude of post-stroke activity occurs in animals with severe brain injury.

Nocturnal activity levels in rhesus macaques at baseline were not substantially different compared to values determined after stroke, although a nearly significant correlation between infarct volume and nocturnal activity was observed (**Fig. 2C**; $p = 0.05$). Mean daily nocturnal activity prior to stroke ranged from 8.6 to 89.0 counts in all male rhesus macaque animals tested (10-fold range), with a mean nocturnal activity value of 29.3 ± 16.4 (mean $\pm$ SD) counts per day for the entire cohort. Similarly, mean daily nocturnal activity after stroke ranged from 11.4 to 54.5 mean daily counts (~5-fold range) with a mean nocturnal activity of 25.3 ± 12.1 (mean $\pm$ SD) counts per day for the entire cohort. Although mean nocturnal activity did not change dramatically following stroke, we noted a proportional increase in nocturnal activity compared to daytime activity. Nocturnal activity accounted for 3-15% of total activity at baseline prior to stroke and 16-39% after stroke in our study cohort. The reason for a perceived proportional increase in nocturnal activity is primarily due to a concomitant decrease in daytime activity. Therefore, an overall decrease in total activity

occurred, mostly comprised of decreased daytime activity, which lead to a concomitant decrease in day:night activity ratio. The ratio of day:night activity correlated significantly with infarct volume (**Fig. 2D**).

We hypothesized that the surgical model itself or the stress involved in the manipulation of the animals would result in measurable changes in activity levels that were unrelated to the severity of stroke. To address this hypothesis, baseline changes in day-to-day activity prior to stroke were determined for each animal and these changes were compared to mean changes in activity among animals demonstrating only minor brain injury defined as infarct volume $\leq 3\%$ of ipsilateral hemisphere. Overall, most animals had stable daily motor activity prior to stroke. Data from the 22 animals included in this analysis revealed that the mean change in baseline day-to-day activity prior to stroke over a prescribed 4-day period was $11\% \pm 6.5\%$ (**Fig. 3**), with individual values ranging from 2 to 25%. In contrast, animals that had very minimal stroke outcomes ranging from 0-3% of hemisphere ($n=4$; $1.8 \pm 1.3\%$ infarct) demonstrated a mean decrease of 51% in total daily activity level (**Fig. 3**), as well as mean decreases of 57% in daytime activity and day:night activity ratio compared to baseline values. Mean change in baseline day-to-day activity prior to stroke was only 9% for this minor stroke outcome group with individual baseline changes ranged from 3-17%. These data suggest that the experimental protocol may result in substantial activity changes irrespective of infarct volume. Importantly though, activity changes in animals with severe stroke outcomes $>3\%$ hemispheric infarct ($n=18$; mean infarct of $23 \pm 2\%$) showed even greater mean decreases in total daily activity (83%; **Fig. 3**), daytime activity (86%), and nocturnal activity (35%) compared to their changes in day-to-day baseline activity prior to stroke ($13 \pm 3\%$ mean total daily activity change). These results argue that the experimental protocol contributes to significant activity changes ($\sim 51\%$), although correlation analyses (**Fig. 2**) show that activity changes beyond that level were indeed correlated with infarct severity.

Changes in Activity Levels Correlate with Neurobehavioral Outcomes

Given proper training and experience of the observer, subjective measurement of neurological deficits using traditional clinical scaling methods can be applied to NHP. In our study, cumulative neurological assessments made over 7 days in rhesus macaques following surgical occlusion revealed that the extent of deficits compared to baseline values were related to infarct severity quantified by MRI at 48 hours (**Fig. 4**). As we previously reported for adult male Indian rhesus macaques [5], infarct volumes in adult male Chinese rhesus macaques correlated with the cumulative neurological scores ($p < 0.001$; **Fig. 4**). Animals having small 0-3% hemispheric infarct had a range of 592-696 cumulative neurological score (score of 700=no deficit) with a group mean score 648 ± 50 , whereas animals with $>3\%$ hemispheric infarct had greater functional neurological deficits with a mean cumulative score of only 203 ± 139 .

While these data validate the use of subjective scoring, these methods are not easily transferred between laboratories and substantial training is required to implement the scoring paradigm. Therefore, we aimed to determine if objective activity measures were related to subjective neurological scores by performing correlation analyses. Indeed, we found that the changes observed in total daily activity levels (**Fig. 5A**), daytime activity levels (**Fig. 5B**), nocturnal activity (**Fig. 5C**) and day:night activity ratio (**Fig. 5D**) correlated with the level of cumulative neurological deficits measured by subjective neurological scores. Of all of the activity parameters measured in this study, nocturnal activity was least affected by infarct severity. In the absence of data points reflective of animals terminated early (100% reduction), nocturnal activity data did not significantly correlate with neurological score ($p=0.3$). All other activity parameters correlated with neurological score irrespective of the

inclusion of data from these animals. This suggests that nocturnal activity may not be significantly affected by extent of infarct or neurological function.

Discussion

Neurological behavior scoring in a nonhuman primate model of experimental stroke requires personnel with special behavioral training, and is a time consuming process. For example, understanding of the typical behavior patterns of individual monkeys and their reactions to certain stimuli allows for a better interpretation of changing behaviors after the onset of brain damage. However, monkeys often mask signs of weakness or physical deficits, an inherent behavior arising from the fear of predation [13]. Thus, assessing motor dysfunction requires astute observation of each animal by a skilled observer. Once a study has begun, it is ideal to have a single “blind” observer perform the neurological scoring. However, large stroke studies may span for several years and thus observer consistency cannot always be guaranteed. These, as well as other factors, highlight the obvious complexities involved in subjective analysis of neurological deficits in nonhuman primates, as well as the need for additional methods to consistently and objectively evaluate functional neurological deficits both within and between laboratories.

Clinical studies employ accelerometer devices worn by patients following stroke, allowing for quantitative comparisons between affected and unaffected limbs [14-16, 4]. In one such study actigraphic recordings of total motor activity were lower on the impaired arm versus non-impaired arm. These data revealed a significant positive correlation between motor activity and neurological scores during the 1st week post-stroke, corresponding to the time when neurological deficits were most pronounced [14]. While not able to directly assess affected limbs in monkeys, we show for the first time that measuring total body activity using neck collars correlates with outcome in our stroke model. We show that activity in the rhesus macaque was dramatically reduced following stroke corresponding to the infarct volume and neurological outcomes using a validated neurological stroke scale. Given the cost of animals and difficulty of the study, several statistical approaches were undertaken to account for animals terminated at day 2 prior to study end. This is a noted limitation of our study as the chosen statistical methods have the potential to confound the data based on their inherent assumptions. However, analysis of the results excluding these animals revealed similar correlations, suggesting that our interpretations largely remain valid.

Notably, all animals in this study showed a decrease in activity following the surgical occlusion procedure regardless of infarct volume or extent of neurological deficit. Animals showing minimal to no infarct and minimal neurological deficits demonstrated an approximate 51% reduction in total activity compared to 10% baseline daily change in activity observed prior to surgical manipulation of these animals. These data suggest that the experimental protocol results in a reduction in total activity regardless of the severity of clinical outcome. These data could also argue that some damage is unidentifiable by MRI, but may contribute to a decline in activity; therefore, a detailed histological examination may be warranted as gross examination of the brain tissue from these animals did not appear noticeably infarcted by day 7 following stroke (data not shown). The status of the brain after longer survival times would likely be informative. Importantly, animals with larger strokes demonstrated much larger decreases in activity, which correlated with infarct severity suggesting that an appropriate dynamic range exists for this outcome variable. Thus, activity parameters measured by actigraphy are useful as an outcome variable in preclinical studies testing novel drug candidates.

Individual differences in daily pattern and level of activity have been readily observed in humans [17] and experimental animals [18, 19]. Nocturnal activity varied significantly

between individual animals in our study. This finding was anticipated because several published studies note a similar 10-fold range in the duration and level of nocturnal activity in both free-ranging male [20] and cage-housed female rhesus monkeys [19]. Another study showed that nocturnal activity was correlated with daytime activity, suggesting that similar mechanisms are operating at both times of day and similar variability exists. This positive correlation between day and nighttime activity is also reinforced by studies in humans [21].

The mechanisms that regulate physical activity are poorly understood although studies show that several neurotransmitters (e.g. serotonin, dopamine, norepinephrine [22]), as well as estrogens [23], have all been shown to regulate physical activity. Moreover, certain brain regions may be involved in activity regulation [22]. Studies of lesioned areas of the basal forebrain, ventromedial hypothalamus, paraventricular nucleus, amygdala, and thalamus suggest that these structures may play a role in regulating activity [22], although these are not areas commonly affected in our model. Recent data show that orexin A injected into the paraventricular nucleus increases both daytime and nocturnal activity in rats suggesting that orexin A may play a role in global activity regulation [24]. It may be fruitful to evaluate the status of neurotransmitters, hormones, and orexin A in the context of stroke in the rhesus macaque and to correlate these values with changes in activity level.

Activity changes due to stroke pathology are but one aspect of this technology. Accelerometry was also recently used as an outcome measure in a human clinical trial in patients with stroke. The effects of indeloxazine hydrochloride and ticlopidine hydrochloride was evaluated in 17 patients with stroke [15]. These data showed that both groups of patients treated with drugs improved significantly after an 8-week administration period compared to the improvement seen in the control group. Our data show that actigraphy may similarly be a useful surrogate marker for treatment efficacy in preclinical stroke studies in nonhuman primates, such that improvement in activity measures could be indicative of improvement in neurological function and a reduced infarct volume, and ultimately drug efficacy. Moreover, temporal changes are often not adequately discerned by subjective neurological scoring performed at limited or prescribed time points, whereas actigraphy could allow for continuous analysis. This method also provides an inexpensive means to follow neurological improvement or decline with minimal expert staff or imaging needs over long periods of time. There could be significant value in the assessment of clinical improvement over time, which may be a component of efficacy with some stroke therapeutic strategies. Human studies evaluating motor and functional recovery patterns after stroke have confirmed the importance of the first month for recovery [25], which is a timeframe easily monitored by these devices.

Conclusions

In this study, we confirm that changes in activity occur following stroke and these changes significantly correlate with severity of stroke measured by infarct volume or neurological score in a rhesus macaque model of cerebral ischemic injury. These data show that objective activity measures can be used to support neurological findings leading to the rapid adaptation of these methods among many laboratories. Additionally, the use of such devices for the preclinical evaluation of therapeutics in a monkey model of stroke has the potential to provide a more extensive assessment of changes in global activity. Studies to validate the use of actigraphy in a preclinical setting by testing the efficacy of novel therapeutics in this rhesus macaque stroke model are underway. These studies should provide more insight as to the benefits of this technology for stroke drug development.

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Fig. 1. Daily schedule and study timeline

A) Daily schedule reflects 24-hour period with hour of day indicated reflecting a 12-hour light-dark cycle wherein black denotes periods of lights out (nocturnal activity) between 1900 h and 0659 h and white denotes periods of lights on (daytime activity) between 0700 h and 1859 h. Daily interactions included morning clinical observations (light gray circle), food administration (star), snacks for enrichment (black dot), and room washing (gray rectangle). **B)** The study timeline illustrates acclimation for at least three weeks prior to stroke. At baseline activity levels from Days -7 to -4 were used to derive a mean baseline activity for each animal for each parameter. After stroke, activity levels from Days 3 to 6 of the study were used to derive individual post-stroke mean activity levels. This time point was selected since animals were being given post-stroke analgesics on study days 1 and 2. Post-stroke values were then compared to baseline mean activity values and the percent change in activity post-stroke was determined for each activity parameter for each animal.

Fig. 2. Correlation between mean activity values versus infarct volume

A) Representative brain images showing infarct regions determined by T2-weighted MRI. Data reflect representative MR images of an animal with a 3% stroke volume (top panel) and 26% stroke volume (bottom panel). The figures on the left of each panel are of a single axial slice collected at the level of the arrow seen on a 3-D representation of each brain (right). Areas of infarct are hyperintense, circumscribed on the axial slices and denoted in red on the 3-D reconstruction. Lines represent the anterior (A)-posterior (P), left (L)-right (R) and inferior (I)-superior (S) axes. **B-E**) Pearson's correlation was performed using **(B)** total activity ($p < 0.0001$), **(C)** daytime activity ($p < 0.0001$), **(D)** nocturnal activity ($p = 0.05$) or **(E)** day:night activity ratio ($p = 0.05$) versus infarct volume measured at 48 hours post-stroke. Data show that all but nocturnal activity correlated with infarct volume. Baseline activity changes (not shown) were not significantly correlated to infarct volume.

Fig. 3. Changes in total daily activity were related to the experimental procedure and correlated with infarct volume

At a fixed timepoint ~1 week prior to stroke following ~2 weeks of acclimation in home cages, the mean change in day-to-day activity over 4 consecutive days was determined for each animal (Baseline). Mean change in total daily activity animals with infarct volumes ≤3% of hemisphere are shown (white bar, n=4), compared to data from animals with >3% infarct (grey bar; n=18). Data reflect mean ± SEM.

Fig. 4. Correlation between total cumulative neurological score and infarct volume
Correlation analysis revealed statistically significant correlation between 7 day cumulative neurological score and infarct volume measured as percent of ipsilateral hemisphere ($p < 0.0001$).

Fig. 5. Correlation between percent change in mean activity counts and cumulative neurological scores

Pearson's correlation analysis revealed statistical significance for **(A)** total activity ($p < 0.0001$), **(B)** daytime activity ($p < 0.0001$) **(C)** nocturnal activity ($p = 0.008$) and **(D)** day:night ratio ($p < 0.0001$) versus mean 7 day cumulative neurological score. Baseline activity changes (not shown) were not significantly correlated to neurological score.