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National Centre for the Replacement, Refinement and Reduction of Animals in Research

The LifeShirt PreClinical System is a significant advance towards answering the call of the 3Rs

Available at www.nc3rs.org.uk

Background

The LifeShirt PreClinical System is a unique technological development that offers the ability to collect high quality respiratory and other data in a non-invasive manner, resulting in significant advancement of both refinement and reduction of the use of animals in research. The System represents a paradigm shift improvement in the data collection and analysis capabilities available to researchers working with laboratory animals. Prior to its development, collection of respiratory data from animals was severely limited without restraining them, which can cause stress and distress, or anaesthetizing them which can confound the data. The collection of quality electrocardiogram (ECG) data required surgical implantation of electrodes or restraint to apply 'alligator clips' to collect a few moments of wake data. Most data collected from animals, particularly from larger species such as non-human primates and dogs, was accomplished via surgically implanted sensors in the abdominal and/or thoracic cavities. This approach has the obvious disadvantage of requiring surgery, which is associated with pain and risk of infection, and a period of recovery. Furthermore, additional surgeries may be required for maintenance and removal of failing implanted devices, for battery replacement and, in some truly undesirable cases, for replacement of sensor units after their removal by non-human primates investigating the foreign body in their body cavity.

The System allows for the collection of very detailed respiratory data, including breath volumes, flow-rates and indicators of changes in respiratory mechanics. This sort of data allows researchers to identify and evaluate the impact of diseases and treatments on the respiratory system in unrestrained animals in a way that has never before been possible. The respiratory signal is derived from respiratory inductance plethysmography (RIP), a technology that has been used widely in the hospital and intensive care unit environment to monitor respiration without the use of a mouthpiece or facemask. The sensor set consists of two bands; one that circles the abdomen and one that circles the thorax. Each sensor provides a detailed waveform reflecting movement of those body compartments that together comprise the chest wall. Collecting data from the distinct thoracic and abdominal compartments provides researchers the opportunity to evaluate the synchronicity of movement in chest and abdomen and thereby make inferences regarding changes in airway resistance and other clinically relevant conditions associated with breathing.

The System also enables researchers to improve their research design and analysis by providing continuous data over extended periods of time. Current research design is based upon the analysis of discrete time points (frequently baseline and end-study). This analysis approach has the risk that significant physiologic changes that occur outside of those discrete periods will be missed. Continuous data allows for the possibility of identifying problems that would otherwise have been missed, as well providing context and interpretation for results in the final analysis. While still somewhat nascent, the transition of data collection and

analysis from a discrete to a continuous data model represents a real and important opportunity for researchers to explore new approaches to understanding disease and treatment in the laboratory environment.

The LifeShirt for human use

The LifeShirt is at the forefront of non-invasive monitoring technologies for humans and animals, with usage ranging from the European Space Agency to the United States military, fire-fighters fighting forest and building fires, professional sports teams, large and small pharmaceutical companies, and a number of contract research organisations and academic laboratories. The rugged and wearable nature of the System has made it an ideal tool for the collection of physiologic data in the 'real world'. Recent studies have included:

- validation of the LifeShirt against a laboratory spirometer during various conditions from rest to maximal treadmill exercise in healthy volunteers, patients with congestive heart failure, and patients with chronic obstructive pulmonary disease (1);
- evaluation of the LifeShirt as a tool to quantify the relationship between respiratory mechanics and the deposition of particulates in the airway during cigarette smoking (2);
- use of the LifeShirt to quantify autonomic dysregulation in paediatric subjects with Rett Syndrome (3);
- use of the LifeShirt's accelerometers to classify movements in patients with Parkinson's Disease (4).

The LifeShirt PreClinical System for animal use

The LifeShirt PreClinical System for animal use is an evolutionary development of the LifeShirt for human use. It is a snugly-fitting garment containing respiratory sensors capable of quantifying breathing frequency, breathing volume and total ventilation. ECG electrodes capture a 1 kHz, modified lead 2 ECG signal, and a tri-axial accelerometer provides detailed information about the body position and activity of the subject. The LifeShirt PreClinical garment with sensors and the small electronics unit are contained in a custom-designed protective jacket, similar to an infusion jacket (Figures 1 and 2). All data are wirelessly transmitted to a central computer for observation in real-time, which greatly enhances the speed, efficiency and quality of research data since there is no need to periodically pause for retrieval and downloading of data from a data logging device. Additional sensors can be added to the core suite of respiratory, cardiac, and activity data-streams as required for specific research questions. Specifically, pulse oximetry and skin temperature can be added as necessary and non-invasive blood-pressure monitoring devices are currently being investigated.

Figure 1. *Cynomolgous monkey and beagle dog wearing the LifeShirt Preclinical data acquisition garment.*

Figure 2. *Cynomolgus monkey wearing the LifeShirt Preclinical data acquisition garment and protective overjacket.*

The System collects data continuously for a minimum of 26-hours and longer studies require only a brief interruption to exchange transmitters. As many as 16 animals can be continuously monitored in a single laboratory (i.e. current radios support 16 independent channels) and data has been collected from group-housed macaques and dogs with essentially no damage to the systems. Continuous data collection of multiple physiologic and activity data streams enables researchers to evaluate data from unrestrained animals with context that hasn't been available with prior systems (e.g. concurrent collection of the accelerometry data with the cardio-respiratory data in freely-moving animals). Animals can locomote, change body position, sleep, eat and perform other natural activities while unrestrained in their cages. Many of these activities have the potential to introduce movement or other artifact into the data if not controlled for, and it is this concern that leads many experiments to be designed around brief data collection periods during which animals are restrained in a sling, restraint chair or other similar type apparatus. With the LifeShirt PreClinical System, animals can be left alone for extended periods of time while multiple physiologic data streams are continuously and synchronously collected. Researchers can then review the data and identify periods of activity and rest, as well as when the animals are adopting certain body positions, to effectively 'control for the uncontrolled' nature of the animal's behaviour while unrestrained.

The LifeShirt PreClinical System and refinement

The technical capabilities of the LifeShirt PreClinical System offer a substantial advance on current data collection methodologies by improving the quality of data collected with the combination of detailed respiratory data and ECG. Moreover, the multiple, synchronous data-streams provide context for the evaluation and interpretation of data, making data collection from unrestrained animals a valid option for researchers designing studies. Finally, the System eliminates the necessity of surgically implanted sensors for the collection of cardiopulmonary data. Thus it can significantly improve the quality of life of animals used in research.

The ability of the LifeShirt PreClinical System to monitor animals for extended periods of time without restraint and in the absence of human monitoring allows for the identification of behavioural and movement patterns that can be an early indication that the animals are starting to demonstrate stereotypies. Interventions can then be enacted early to help preserve the animals' wellbeing. For example, it has been observed by some researchers using the System that a non-human primate that was performing stereotypical behaviour had a very repetitive movement pattern on the accelerometer, as well as very little physiologic 'rest' during the lights out period. While it certainly cannot be posited that this is THE mark of stereotypical behaviour, regular monitoring with LifeShirt PreClinical System allows animal care teams to observe changes in behavioural and activity patterns that are indicators of stress levels becoming problematic. These observations can then be used to trigger modifications to the caging, stimulation, and socialization of the animals as necessary.

Work is continuing to develop additional novel endpoints with the System. These range from the development of a cough identification algorithm which could be used to evaluate novel vaccines for kennel cough, to post-myocardial infarct respiratory and ECG changes, to respiratory patterns characteristic of asthmatic bronchoconstriction.

The LifeShirt PreClinical System and reduction

In the safety pharmacology and pharmaceutical toxicology research environments, cardiac and pulmonary studies are often conducted independently. The LifeShirt PreClinical System allows for these studies to be conducted concurrently, thus permitting a reduction in the number of discrete experiments and hence the number of animals required for these programs. Addition of the System to toxicology studies will enable the collection of respiratory data at an earlier stage of drug development than is current practice. Typically, respiratory data is not collected during the toxicology phase of drug development, but could be collected simultaneously to ECG and QT interval analyses, thus expediting the entire data collection period. This has the potential advantage of screening out molecules with an undesirable respiratory effect at an earlier stage, thus reducing the number of molecules carried forward into safety pharmacology testing, and hence reducing further animal use.

Data samples

LifeShirt PreClinical data has been presented at meetings of the Society of Toxicology (2005, 2006, 2007), Safety Pharmacology Society (2006, 2007), and American Association for Laboratory Animal Science (2005, 2006) by researchers working in the academic, drug development, and contract research environments (5-12) These data are currently being developed into manuscripts. In general, the LifeShirt data has been shown to agree well with established technologies such as a pneumotachograph for quantification of breathing frequency and tidal volume, and implanted devices for heart rate and ECG, and has been shown to be sensitive to changes associated with standard positive controls (methacholine, morphine, hypercapnia, caffeine).

A sample of the LifeShirt respiratory data from an unrestrained, awake cynomolgus monkey is shown in Figure 3. The tidal volume trace (Vt) is a weighted sum of the ribcage (RC) and abdominal (AB) signals. Figure 4 shows a 5 minute sample of data from an unrestrained, sleeping beagle. The light green bands mark two apneic periods, each of approximately 20 seconds in duration. The third trace shows beat-to-beat heart rate (HR) and demonstrates the substantial heart rate variability characteristic of the strong vagal tone in beagles. The large peak-to-trough variability in the HR trace is consistent with respiratory sinus arrhythmia which causes heart rate to accelerate during inspiration and slow during expiration. This variability can be observed in the 'accordion' like pattern of the raw ECG trace. During the apneas, HR becomes very stable as the impact of pressure fluctuation in the thorax is absent. ECG during these apneic periods represents a 'pure' ECG, in that the morphology of the wave during the apnea reflects the electrochemical activity of the myocardium absent the respiratory influence. Combining the ECG and respiratory signals allows these periods to be identified and may offer researchers an opportunity to isolate these periods for analysis of QT-interval, for example, in the future without having to attempt to account for the constantly changing heart rate during normal breathing. Finally, Figure 5 shows a sample of telemetrically captured 1-kHz ECG acquired from an unrestrained, awake cynomolgus monkey with surface electrodes; the resolution of the ECG is clean and of excellent resolution.

Animal welfare considerations

The LifeShirt PreClinical System appears to be well tolerated by both monkeys, including cynomolgus monkeys, rhesus macaques and African green (vervet) monkeys, and dogs, including beagles, foxhounds and mongrels. Data collection does require that the animal subjects be fitted with a snug fitting garment and, for unrestrained use, a protective overjacket. The application of these garments has generally not caused difficultly but it is prudent to allow a period of habituation. A recent poster from Erwin et al. (12) has reported elevated blood pressure and heart rate for cynomolgus monkeys naïve to jacketing, with either the LifeShirt alone or a protective jacket alone. Erwin et al. raise to the attention of the research community the importance of habituating non-human primates to any data collection instruments, as these animals are sensitive to any disruption in their normal routine. There have been no reports of non-human primates or dogs distressed while wearing the LifeShirt PreClinical System, and there have been a number of observations reporting heart rate in the normal range and unchanged from the period prior to or following the LifeShirt garmented session.

Every attempt has been made to ensure that the LifeShirt PreClinical System garments are as comfortable as possible, including padding of contact points and covering of friction points on the protective jackets with a soft, silky material. Despite this, it is possible for animals to experience some skin irritation at the site of ECG electrode attachment and at other pressure/friction contact points. However, one advantage of the LifeShirt PreClinical garment design is that it can be continually modified in response to customer feedback and efforts are being continually made to improve comfort.

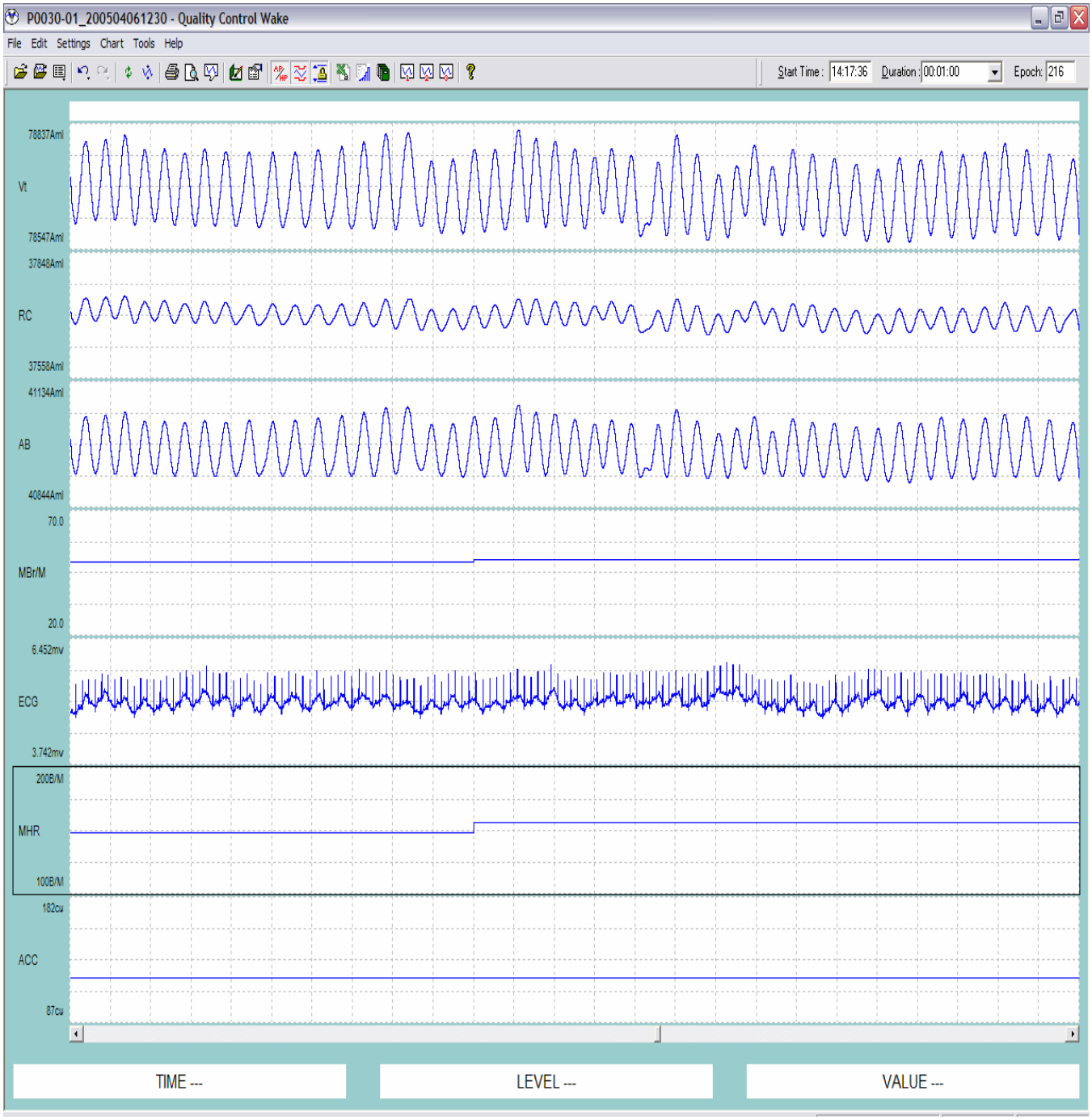

Figure 3. *1:00 minute sample of data from an unrestrained, awake non-human primate..*

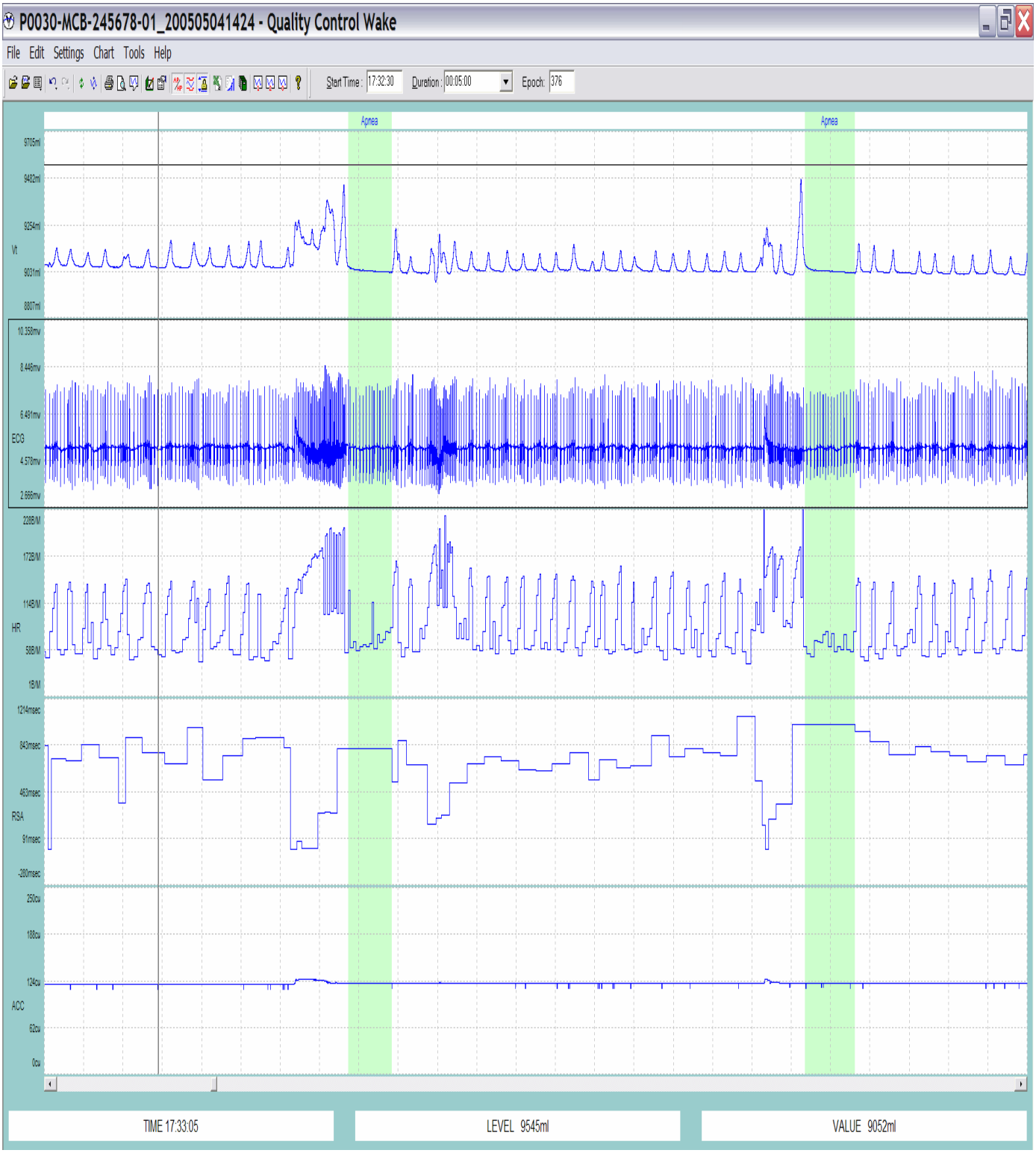

Figure 4. *5:00 minutes of data from an unrestrained, sleeping beagle. Note the apneas (green) and significant heart-rate variability indicative of the substantial vagal tone in the beagle.*

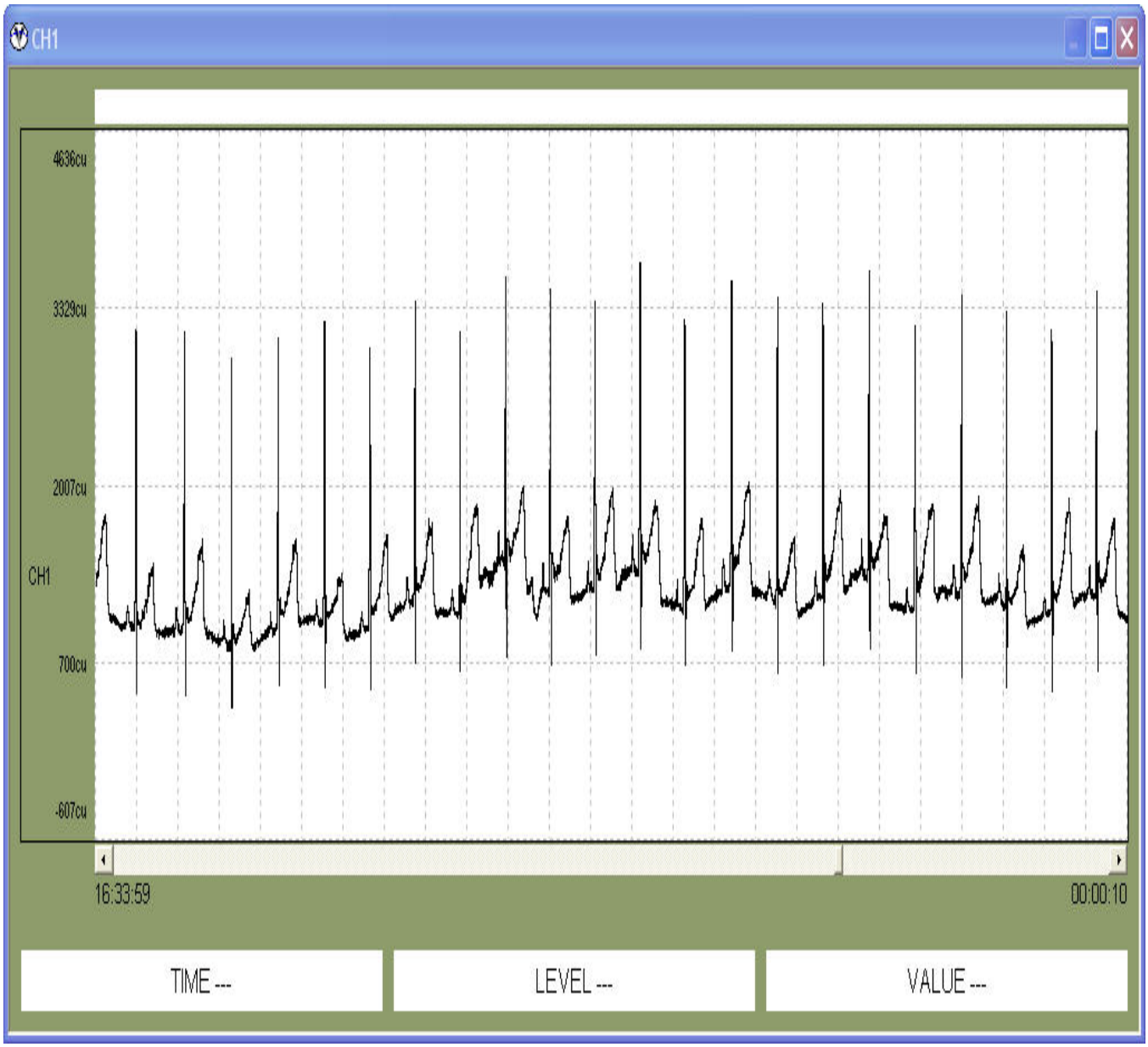

Figure 5. *10 second sample of 1-kHz ECG collected telemetrically from an unrestrained non-human primate*

Future developments

The LifeShirt PreClincial data acquisition garment is currently being modified for work with additional species, including minipigs, large pigs, horses and additional species of non-human primate. Battery life is also continually being extended and additional data-streams are being added. Preliminary data has been very encouraging with pulse oximetry, and VivoMetrics has identified a non-tail-cuff, non-invasive, beat-by-beat blood pressure monitor that has been very encouraging in initial trials with non-human primates.

Conclusions

The LifeShirt PreClinical System represents a significant advance in animal welfare in the research environment. While not enabling the replacement of animals in experiments, the System does offer improvements in terms of both refinement and reduction of the use of animals in research. The most significant improvements available for the 3Rs in the near term will be accomplished via developments in technology and the exploration of novel approaches to data collection and analysis. The LifeShirt PreClinical System represents a breakthrough in both of these areas.

Glossary

Accelerometer: Electromechanical device for identifying body position and activity.

Apnea: Temporary absence or cessation of breathing.

Respiratory Inductance Plethysmography: Quantification of respiratory variables by means of a device to quantify changes in external chest-wall volume.

Plethysmograph: Instrument for recording variations in volume of an organism, organ, body-part, or limb.

Pneumotachograph: Apparatus for recording the rate of airflow to and from the lungs.

Pulse oximetry: Non-invasive method of monitoring of the percent of red blood cells which have bound oxygen.

Spirometer: Apparatus for measuring the volume of air inspired and expired by the lungs.

Tri-axial: Having or involving three axes.

Vagal tone: Apparent influence of the vagus nerve producing a slow and irregular heartbeat which varies across the respiratory cycle.

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