Original article

Combined cardiopulmonary assessments with implantable telemetry device in conscious freely moving cynomolgus monkeys

S. Authier [a,b,*], Paul Haefner [c], S. Fournier [a], E. Troncy [b], L.B. Moon [c]

[a] LAB Research Inc., 445 Armand Frappier, Laval, Quebec, Canada H7V 4B3
[b] GREPAQ, Department of Veterinary Biomedicine, Faculté de médecine vétérinaire, Université de Montréal, P.O. box 5000, St-Hyacinthe, Quebec, Canada J2S 7C6
[c] Data Science International, 119 14th Street NW, St. Paul, MN 55112, USA

ABSTRACT

Female cynomolgus monkeys were surgically implanted with telemetry transmitters recording ECG (DII), arterial pressure, physical activity, body temperature, and tidal volume. Respiratory rate (RR) and tidal volume (TV) were monitored simultaneously with the telemetry transmitter using impedance. Impedance-based monitoring of RR and TV by telemetry correlated with controlled TV and with pneumotachometer (>98%) in restrained animals. Control drugs with cardiovascular and respiratory effects, including saline, medetomidine (0.01, 0.02 and 0.04 mg/kg) and cocaine (0.5, 1.0 and 1.5 mg/kg) were administered intravenously. An averaging epoch of 5 min was used for analysis of respiratory data. Medetomidine induced significant respiratory depression with decrease in RR and TV in freely moving animals while cocaine increased TV, RR and minute ventilation (MV) with concomitant increase in heart rate when compared with time matched values from saline-treated animals. The onset, duration and magnitude of cardiovascular and respiratory changes were correlated. This highlights the dependency of the cardiovascular and respiratory systems. The use of cardiopulmonary monitoring can allow continuous monitoring including during night time when variability of respiratory parameters is lower. Monitoring of cardiovascular and respiratory parameters in the same animals could also help to decrease the number of animals used in research.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Respiratory safety pharmacology is most often performed in conscious rats (Lindgren et al., 2008). The ionic mechanisms of repolarization in rats differ from larger species, including humans. Hence, large laboratory animals are most frequently used for cardiovascular safety pharmacology (Lindgren et al., 2008) to evaluate test article effects on ventricular repolarization *in vivo* and more specifically inhibition of the hERG (I_{Kr}) potassium channel as defined in the S7B guideline (U.S. FOOD AND DRUG ADMINISTRATION, 2005). As a result, combined cardiovascular and respiratory safety pharmacology can be conducted in large laboratory animals.

As per S7A guideline, respiratory safety pharmacology should include *respiratory rate and other measures of respiratory function (e.g. tidal volume or hemoglobin oxygen saturation)* (U.S. FOOD AND DRUG ADMINISTRATION, 2001). The ability to monitor multiple physiologic systems such as electrocardiography, hemodynamics, temperature and respiration in a single implantable telemetry device allows cardiopulmonary safety pharmacology investigations. Potential benefits include integrated interpretation of pharmacological responses with possible correlation between cardiovascular and respiratory effects. A methodology with quantitative cardiopulmonary monitoring could also support reduction and refinement (from the 3Rs) in animal experimentation. Lastly, combining cardiovascular and respiratory safety investigations could improve resource allocation and timelines which are often crucial in drug development. The current study undertook preliminary evaluation of implantable telemetry devices with ECG, arterial pressure, respiratory rate (RR) and tidal volume (TV) monitoring in conscious cynomolgus monkeys. Safety pharmacology uses constantly evolving methodologies to inform on the risk benefit of drug candidates and encompasses regulatory and scientific considerations (Pugsley, Authier, & Curtis, 2008). Respiratory monitoring in large laboratory animals is often conducted with methodologies that require restraining (Authier, Legaspi, Gauvin, & Troncy, 2009). The S7A guideline further specifies that: "Data from unrestrained animals that are chronically instrumented for telemetry, data gathered using other suitable instrumentation methods for conscious animals, or data from animals conditioned to the laboratory environment are preferable to data from restrained or unconditioned animals." In 2009, six biological license applications (BLAs) were approved by the Food and Drug Administration which is just one less than the new century record of seven achieved in 2002 (Sheridan, 2010). Non human primates are often required during non-clinical development of biologics and the current technology could facilitate of safety pharmacology study conduct in this species.

* Corresponding author. LAB Research Inc., 445 Armand Frappier, Laval, Canada H7V 4B3. Tel.: +1 450 973 2240x1403; fax: +1 450 973 2259.
E-mail address: authiers@labresearch.com (S. Authier).

2. Materials and methods

2.1. Statement on use and care of animals

During the study, care and use of animals were conducted in accordance with principles outlined in the current Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). LAB Research Inc.'s facility is AAALAC accredited. All procedures were conducted as per Standard Operating Procedures (SOPs) in place.

2.2. Animal housing

The animal room environment was controlled (temperature $21 \pm 3\ °C$, humidity 30–70%, 12 h light, 12 h dark, 10–15 air changes per h) and temperature and relative humidity were monitored continuously. A standard certified commercial primate chow (Certified Primate Diet 2055C™, Harlan Teklad, Madison, WI, USA) was available to each monkey twice daily.

2.3. Animal preparation

Five (5) cynomolgus (*Macaca fascicularis*) monkeys (ages: 3 to 5 yrs, wt.: 2.8–3.8 kg) were surgically prepared with telemetry transmitters (prototype TL11M3-D70-PCTR, DSI, St-Paul, MN, USA) at LAB Research Inc.. Prophylactic antibiotics (penicillin 20 000 IU/kg) were administered by intramuscular (IM) injection at least 30 min prior to surgery and once a day for at least 48-hours post-surgery. Preemptive analgesia (buprenorphine, Temgesic™, 0.05 mg/kg, Schering-Plough, Welwyn Garden City, Hertfordshire, UK) was administered by IM injection before surgery and every 6 to 12 h for at least 3 days post-surgery. Animals were placed on a heating pad and inhaled a mixture of oxygen (O_2) and isoflurane (AErrane™, Baxter Corporation, Mississauga, ON, CAN) with the O_2 flow meter and the vaporizer set at 1.0 L/min, and 2.0%, respectively. Spontaneous breathing was used except for animals presenting SpO2 lower than 85% in which case, RR was maintained at 15 breaths/min with an inspiratory airway pressure between 18 and 24 cm H_2O using a mechanical ventilator (2002, Hallowell EMC, Pittsfield, MA, USA). During anesthesia, monitoring included heart rate and pulsatile hemoglobin saturation in O_2 (VetOx 4404™ pulse oximeter, Heska, Fribourg, Switzerland). An abdominal midline skin incision was initially done cranial to the umbilicus and a longitudinal incision was done (length of approximately 4 cm) in the middle of the *rectus abdominis* muscle (parallel to muscle fibers). The telemetry transmitter was placed between the *internal abdominal oblique* muscle and the aponeurosis of the *transversus abdominis* muscle. ECG electrodes (DII) and arterial pressure lines (right femoral artery) were tunnelled subcutaneously to a small skin incision and implanted as previously described (Authier, Tanguay, Gauvin, Fruscia, & Troncy, 2007). A femoral vein catheter (3 Fr–6 Fr Cath-in-Cath™, AVA Biomedical, Chicago, Illinois, USA) was surgically implanted for dosing of freely moving animals.

2.4. Experimental surgical method

A pair of impedance leads was implanted subcutaneously on the midplane between the sternum and the spine at the mid thorax level. The impedance leads were identical to standard ECG leads and were attached in a common fashion to the intercostal tissue by creating a loop from approximately 2–3 cm of exposed lead coil. The *rectus abdominis* was sutured with a simple continuous suture. All skin incisions were closed with interrupted buried sutures (Polyglactin 3-0, Vicryl™, Ethicon Inc., Somerville, New Jersey, USA). Two pairs of impedance leads were implanted subcutaneously with each pair placed bilaterally on the thorax at the midplane between the sternum and the spine at the mid thorax level (xiphoid process). The impedance leads were identical to the standard ECG leads and were attached in a common fashion to the intercostal tissue by creating a loop from approximately 2–3 cm of exposed lead coil and with approximately 2–3 cm of distance between each electrode in a pair. The *rectus abdominis* was sutured with a simple continuous suture. All skin incisions were closed with interrupted buried sutures (Polyglactin 3-0, Vicryl™, Ethicon Inc., Somerville, New Jersey, USA).

2.5. Experimental methods

Respiratory monitoring was initiated within 24 h following surgery. Telemetry data was collected with Data Science International DataQuest ART v4.2. Signal analysis was conducted using Ponemah v4.9 sp1. The impedance signal was calibrated to flow using simultaneous monitoring by the telemetry implant and a pneumotachometer (Model 8420 with a 5 L/min capacity, Hans Rudolph, MO, USA) and Validyne differential pressure transducers (Model DP45, ± 2.25 cm H_2O).

2.5.1. Controlled ventilation

Approximately 3 weeks after the telemetry implantation, non human primates were anesthetized with an intravenous bolus of propofol (6 mg/kg) and anesthesia was maintained with a continuous infusion of propofol (42 mg/kg/h). After endotracheal intubation, animals were ventilated with 15 cycles of controlled TVs at 20, 40, 60 and 80 mL. Volumes of 60 and 80 mL were only used when peak inspiratory pressure was lower than 25 cm H_2O. TVs were calculated using Ponemah v4.9 sp1. Quality control of breath-to-breath marks was performed by a trained veterinarian for all respiratory cycles included in the analysis. Correlation between both modalities was assessed with the Pearson correlation factor (R^2).

2.5.2. Comparison of impedance-based monitoring with a reference modality

Non human primates were restrained in a chair equipped with a helmet and a bias airflow. TV and RR were monitored simultaneously with a pneumotachometer (Model 8420 with a 5 L/min capacity, Hans Rudolph, MO, USA) and Validyne differential pressure transducers (Model DP45, ± 2.25 cm H_2O) and with impedance using telemetry. Flow signals were conditioned using Data Science International ACQ7700 Carrier signal conditioner amplifier (Model: 13-7715-35). Remifentanil was administered intravenously (1.688, 3.375 and 6.75 µg/kg) to modulate RR and TV.

2.5.3. Respiratory monitoring in freely moving animals using implantable telemetry

Control drugs {saline, medetomidine (0.01, 0.02 and 0.04 mg/kg) and cocaine (0.5, 1.0 and 1.5 mg/kg)} were administered to four (4) non human primates with a 24 h continuous monitoring period. Animals were initially dosed during a short restraining period via a temporary catheter (Surflo® 24G, Terumo®, Elkton, Maryland, USA) placed in the cephalic vein. Then, animals were equipped with a tether and a jacket and were administered medetomidine and cocaine without any restraining via the Cath-in-Cath™ (AVA Biomedical, Chicago, Illinois, USA).

2.6. Data analysis

Respiratory signal analysis was conducted using Ponemah v4.9 sp1. and analysis parameters were adjusted to provide automated breath detection. Accuracy and correlation were evaluated by linear regression comparing reference measurement modalities with impedance-based telemetry monitoring. Analysis of variance (ANOVA)

for repeated measures was used when appropriate. When applicable, data was presented as mean with standard error of mean (SEM).

3. Results

Tidal respiratory patterns were observed with the impedance-based respiratory monitoring (Fig. 1). Pearson correlation factors (R^2) between controlled TVs (controlled using syringes in intubated animals) and impedance-based respiratory monitoring obtained animals were >98% in anesthetized animals. Correlation between respiratory monitoring with a pneumotachometer with a bias flow helmet and implantable telemetry (1 min averages) in conscious restrained animals was also >98% (Fig. 2).

Pharmacological effects of remifentanil including decreased minute volume (MV), apnea and a compensatory increase in MV were identified with the pneumotachometer and with impedance monitored by telemetry (Fig. 3.). To illustrate the correlation between the cardiovascular and pulmonary systems, heart rate and impedance-based MV presented a correlation of 82% (Fig. 4) during the 24-hour monitoring following saline administration. Medetomidine (0.04 mg/kg) induced a significant decrease in TV while cocaine (1.5 mg/kg) induced a significant increase in TV in freely moving monkeys (Fig. 5). The magnitude of respiratory effects following medetomidine and cocaine on MV was dose dependant (Fig. 6). Heart rate changes following medetomidine and cocaine administration were comparable to the effects observed on TV and MV which illustrates cardiovascular dependency (Fig. 7). Cardiovascular and respiratory responses with medetomidine and cocaine administered IV to restrained animals immediately followed by freely moving telemetry monitoring (Figs. 5–7) presented an initial increase in cardiovascular and respiratory parameters due to manipulation.
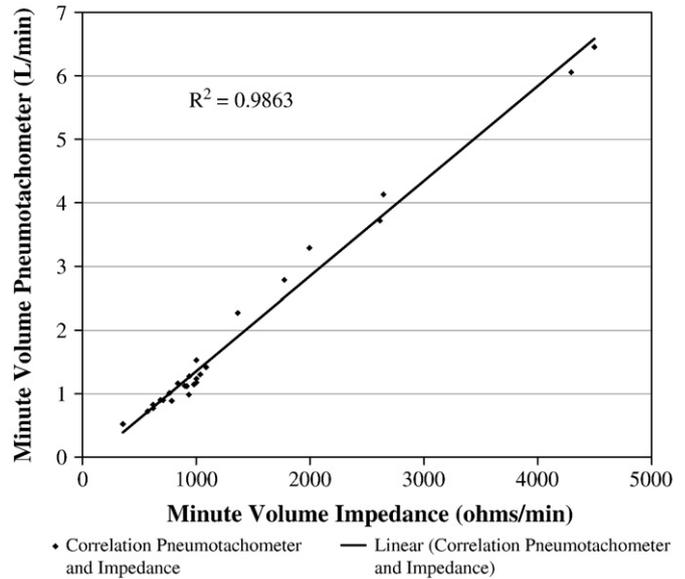

Fig. 2. Correlation between respiratory monitoring using spirometry (pneumotachometer) and implantable telemetry using impedance (1 min average) in a conscious and restrained animal. Restraining was needed for monitoring with the pneumotachometer using a helmet with a bias air flow.

Medetomidine administered to freely moving monkeys (jacket and tether) resulted in a dose-dependent decrease in TV and MV (Fig. 8). Cocaine administered to unrestrained animals induced a significant increase in TV at 1 mg/kg and an increase in RR at 1.5 mg/kg (Fig. 9). This initial increase was not observed in freely moving
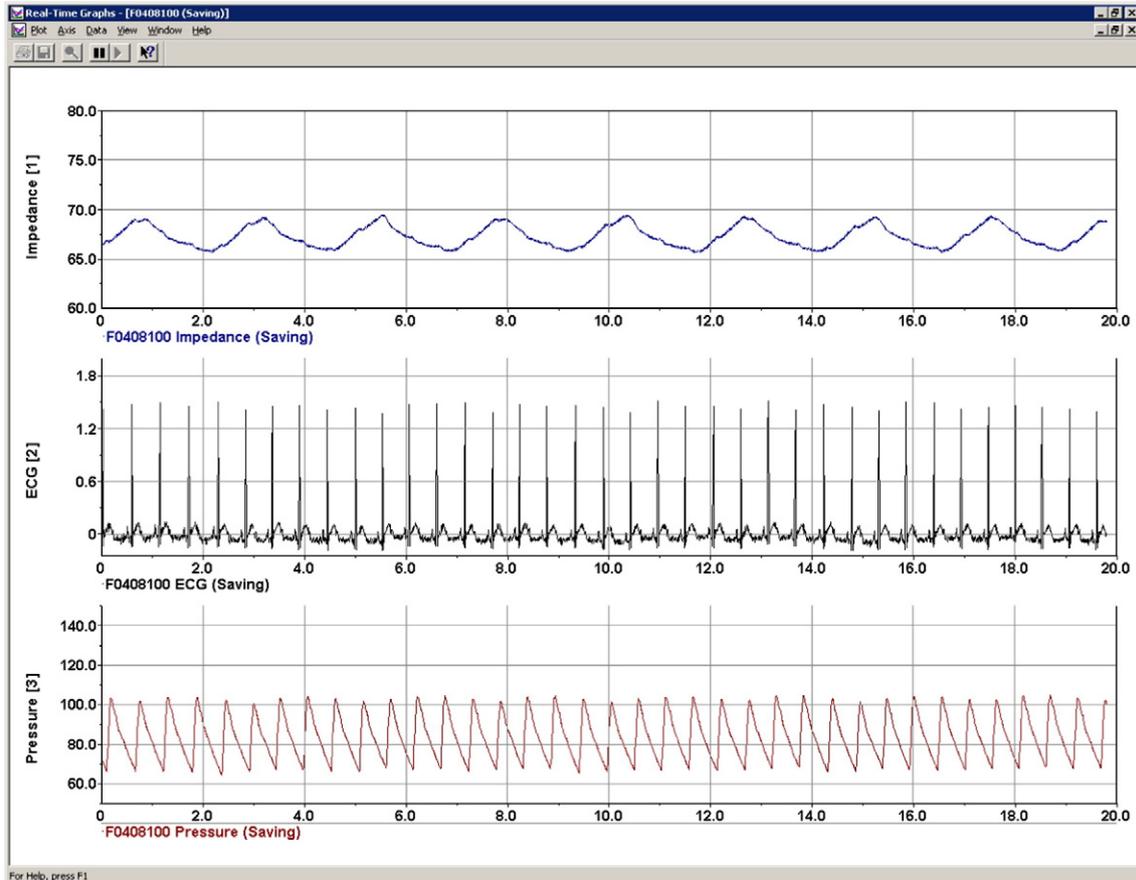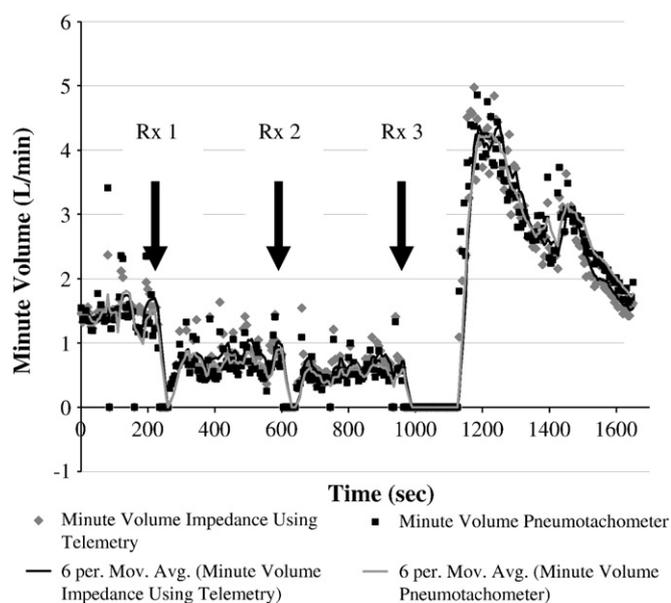

Fig. 1. Graph of impedance-based respiration, ECG, and arterial blood pressure of a non human primate 9 days post implant.
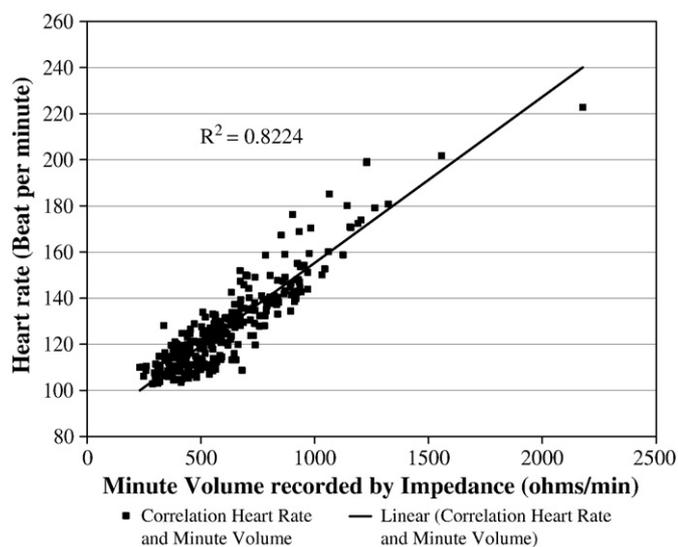
Fig. 3. Simultaneous respiratory monitoring with pneumotachometer and implantable telemetry after IV administration of remifentanil (Rx 1 = 1.688 μg/kg, Rx 2 = 3.375 μg/kg and Rx 3 = 6.75 μg/kg) in a conscious and restrained animal. Both monitoring modalities captured decreased MV, apnea (after Rx 3) and compensatory increase in MV.
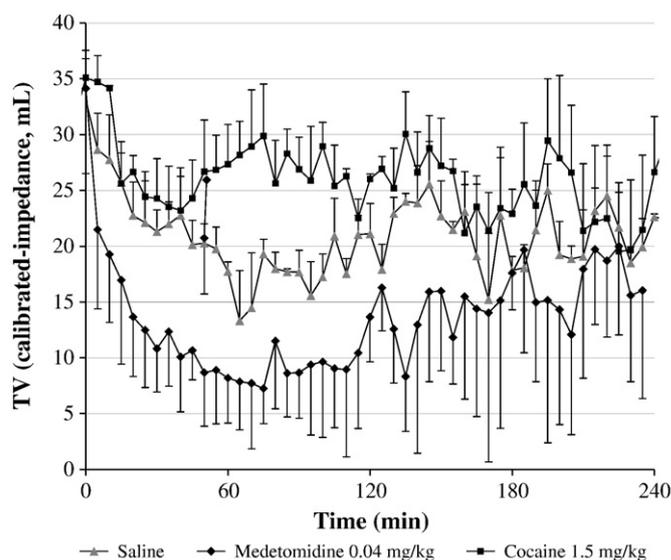
dose administration with permanent femoral catheter, jacket and tether.

4. Discussion

Impedance for measurement of respiratory function has been extensively described in humans (Mond, Strathmore, Kertes, Hunt, & Baker, 1988; Jordaens, Berghmans, Van Wassenhove, & Clement, 1989; Duru et al., 2000) with mean correlation reported between impedance measured MV and direct measures of 0.96% +/−0.03% (Simon et al., 2003). Impedance measurement of MV is commonly used for pacemaker rate adjustment in patients (Cole et al., 2001) based on the assumption that an increased MV reflects an increase oxygen consumption which should be paralleled by an increased
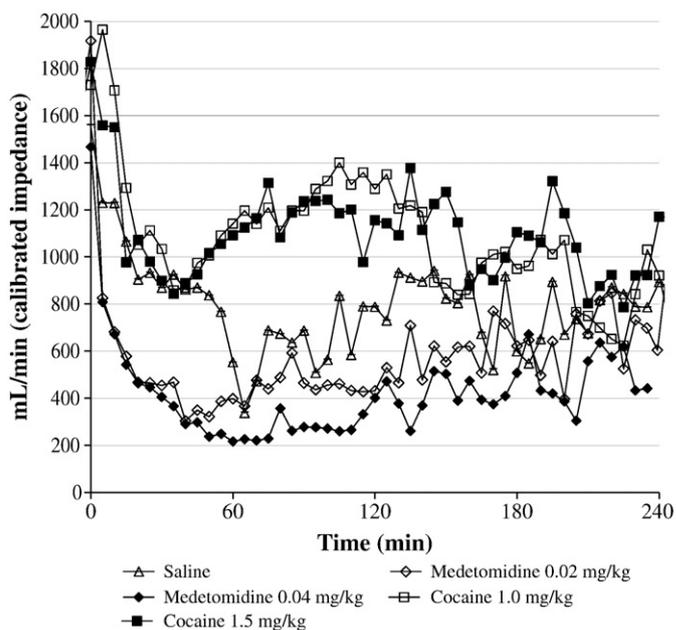

Fig. 5. Effect of medetomidine (0.04 mg/kg) and cocaine (1.5 mg/kg) IV on TV in freely moving cynomolgus monkeys (n = 4). Administration was done in restrained animals which were immediately returned to their cages. ANOVA overall group differences $p < 0.01$.

heart rate. In the safety pharmacology arena, the cynomolgus monkey is an accepted model for non-clinical cardiovascular investigations (Authier, Tanguay, Gauvin, Di Fruscia, Fournier, Chaurand et al., 2007; Authier, Tanguay, Gauvin, Fruscia, & Troncy, 2007; Lynch et al., 2008; Bass, Hanson, & Jackson, 2009) and it is also considered as an acceptable species for respiratory safety pharmacology (Authier et al., 2009). The current study evaluated impedance measured with implantable telemetry for respiratory safety pharmacology investigations.

Correlation between controlled volumes (using a syringe) or pneumotachometer measurements and respiratory monitoring from impedance obtained from implanted telemetry transmitters were comparable to previously reported correlations (Simon et al., 2003).

Fig. 4. Correlation between MV and heart rate over a 24-hour period in freely moving cynomolgus monkeys (n = 4) without pharmacological intervention. The graph illustrates cardiopulmonary dependency as an increase in heart rate is expected to be associated with an increase in MV.
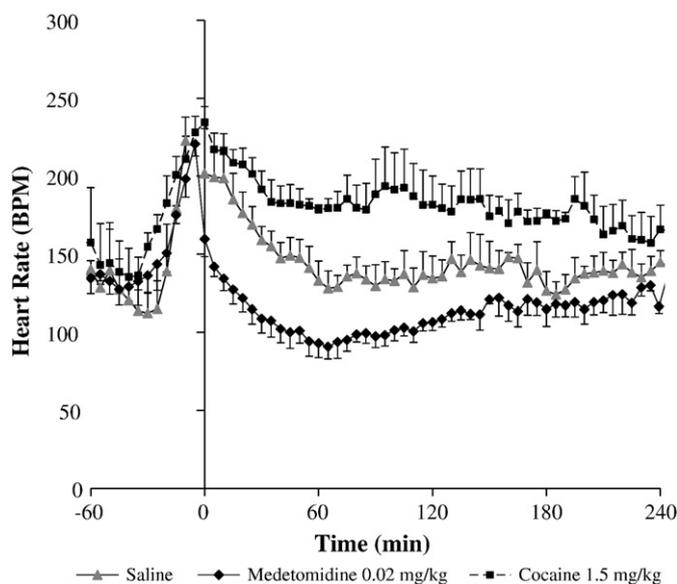


Fig. 6. Effect of medetomidine (0.02 and 0.04 mg/kg) and cocaine (1.0 and 1.5 mg/kg) IV on MV in freely moving cynomolgus monkeys (n = 4). Administration was done in restrained animals which were immediately returned to their cages. ANOVA overall group differences $p < 0.01$.

Fig. 7. Effect of medetomidine (0.02 mg/kg) and cocaine (1.5 mg/kg) IV on heart rate in freely moving cynomolgus monkeys (n = 4). Administration was done in restrained animals that were immediately returned to their cages. ANOVA overall group differences $p < 0.01$.

In freely moving animals, stimulatory effects of cocaine on the cardiovascular and respiratory systems were identified by impedance-based TV and RR monitoring. The duration of cardiovascular and respiratory effects correlated with cocaine pharmacokinetic measured by our group in cynomolgus monkeys at the same doses (unpublished data). Medetomidine was reported to have significant cardiovascular effects in cynomolgus monkeys including prolonged hypotension (Authier, Tanguay, Gauvin, Di Fruscia, Fournier, Chaurand et al., 2007; Authier, Tanguay, Gauvin, Fruscia, & Troncy, 2007). Sedative effects of medetomidine also decreased TV and RR at the doses that were used in the current study. Cardiovascular and respiratory effects of medetomidine showed comparable profiles and were consistent with pharmacodynamic effects of this alpha$_2$-agonist drug. Pharmacological effects observed with positive control drugs in the current study confirmed the ability of the new transmitters to detect drug-induced cardiac and respiratory changes in freely moving cynomolgus monkeys. An initial increase in cardiovascular and respiratory
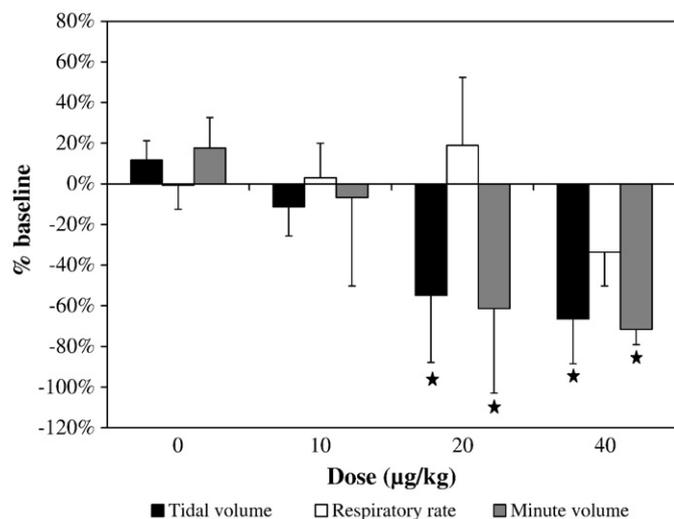
Fig. 8. Dose-dependant response to medetomidine administered IV without restraining (jacket and tether) on respiratory parameters in freely moving cynomolgus monkeys (n = 4). *$p < 0.01$.
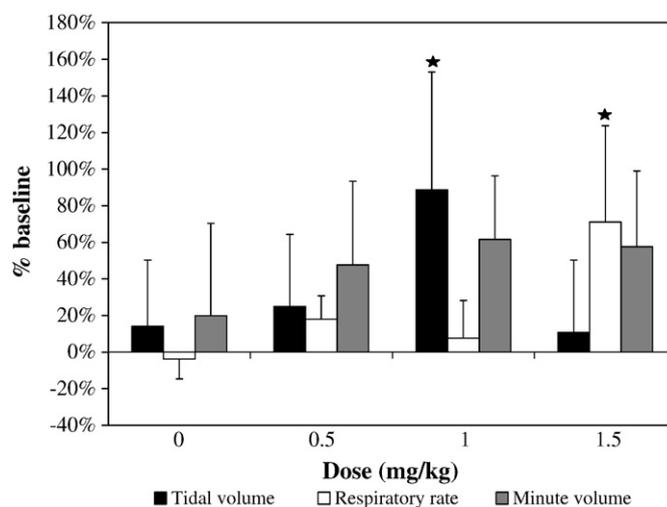
Fig. 9. Increase in TV and RR measured by implantable telemetry following cocaine administered IV without restraining (jacket and tether) in freely moving cynomolgus monkeys (n = 4). *$p < 0.05$.

parameters was noted when animals were restrained for dose administration. When evaluating test articles with rapid exposure (e.g. IV or subcutaneous administration) and short half-lives {e.g. less than thirty (30) minutes}, the use of methodologies that enable dosing in freely moving animals appears indicated.

The monitoring modality evaluated in the current study provided continuous monitoring which generates considerable amount of data for analysis compared with measurement a discrete time points. Continuous monitoring often requires the use of data averaging to facilitate pharmacological interpretation and enables repeated measures analysis of variance (ANOVA) or analysis of covariance (ANCOVA) on selected time points. The power of a statistical analysis plan decreases with the number of time points tested (Sethuraman, & Sun, 2009) which should be considered in appropriate selection of the averaging epoch for data analysis. A 5 min epoch for averaging of non human primate respiratory data in safety pharmacology has previously been reported by our group (Authier et al., 2009) given the intrinsic level of respiratory parameter variability. Cardiovascular and respiratory systems show circadian rhythms with reduced variability during night time (Ando et al., 2005). The reduced parameter variability during night time could translate into increased sensitivity to detect respiratory effects and provide a potential advantage of continuous monitoring compared with assessments at discrete time points.

In conclusion, our results support that impedance measured by telemetry correlate with reference respiratory monitoring modalities. Impedance-based monitoring of TV and RR enabled identification of drug-induced (stimulatory and depressant) respiratory effects in the conscious freely moving cynomolgus monkey. This technology allowed interpretation of cardiovascular and respiratory effects measured concurrently in the same animals.

References

Ando, K., Hombo, T., Kanno, A., Ikeda, H., Imaizumi, M., Shimizu, N., Sakamoto, K., Kitani, S., Yamamoto, Y., Hizume, S., Nakai, K., Kitayama, T., & Yamamoto, K. (2005). QT PRODACT: In vivo QT assay with a conscious monkey for assessment of the potential for drug-induced QT interval prolongation. *Journal of Pharmacological Sciences, 99*, 487−500.

Authier, S., Legaspi, M., Gauvin, D., & Troncy, E. (2009). Respiratory safety pharmacology: Positive control drug responses in Sprague–Dawley rats, Beagle dogs and cynomolgus monkeys. *Regulatory Toxicology & Pharmacology, 55*, 229−235.

Authier, S., Tanguay, J. F., Gauvin, D., Di Fruscia, R., Fournier, S., Chaurand, F., & Troncy, E. (2007). A cardiovascular monitoring system in conscious cynomolgus monkeys for regulatory safety pharmacology. Part 1: Non-pharmacological validation. *Journal of Pharmacological & Toxicological Methods, 56*, 115−121.

Authier, S., Tanguay, J. F., Gauvin, D., Fruscia, R. D., & Troncy, E. (2007). A cardiovascular monitoring system used in conscious cynomolgus monkeys for regulatory safety pharmacology. Part 2: Pharmacological validation. *Journal of Pharmacological & Toxicological Methods*, *56*, 122−130.

Bass, A. S., Hanson, L. A., & Jackson, T. A. (2009). Measuring the risk of torsades de pointes: Electrocardiographic evaluation of PNU-142093 in conscious cynomolgus non-human primates using restraint and non-restraint procedures. *Journal of Pharmacological & Toxicological Methods*, *60*, 51−57.

Cole, C. R., Jensen, D. N., Cho, Y., Portzline, G., Candinas, R., Duru, F., Adler, S., Nelson, L., Condie, C., & Wilkoff, B. L. (2001). Correlation of impedance minute ventilation with measured minute ventilation in a rate responsive pacemaker. *Pacing & Clinical Electrophysiology*, *24*, 989−993.

Duru, F., Radicke, D., Wilkoff, B. L., Cole, C. R., Adler, S., Nelson, L., Jensen, D. N., Strobel, U., Portzline, G., & Candinas, R. (2000). Influence of posture, breathing pattern, and type of exercise on minute ventilation estimation by a pacemaker transthoracic impedance sensor. *Pacing & Clinical Electrophysiology*, *23*, 1767−1771.

Jordaens, L., Berghmans, L., Van Wassenhove, E., & Clement, D. L. (1989). Behavior of a respiratory driven pacemaker and direct respiratory measurements. *Pacing & Clinical Electrophysiology*, *12*, 1600−1606.

Lindgren, S., Bass, A. S., Briscoe, R., Bruse, K., Friedrichs, G. S., Kallman, M. J., Markgraf, C., Patmore, L., & Pugsley, M. K. (2008). Benchmarking safety pharmacology regulatory packages and best practice. *Journal of Pharmacological & Toxicological Methods*, *58*, 99−109.

Lynch, J. J., III, Wilson, A. W., Hernandez, L. E., Nelson, R. A., Marsh, K. C., Cox, B. F., & Mittelstadt, S. W. (2008). Dose–response effects of sotalol on cardiovascular function in conscious, freely moving Cynomolgus monkeys. *British Journal of Pharmacology*, *154*, 1439−1445.

Mond, H., Strathmore, N., Kertes, P., Hunt, D., & Baker, G. (1988). Rate responsive pacing using a minute ventilation sensor. *Pacing & Clinical Electrophysiology*, *11*, 1866−1874.

Pugsley, M. K., Authier, S., & Curtis, M. J. (2008). Principles of safety pharmacology. *British Journal of Pharmacology*, *154*, 1382−1399.

Sethuraman, V., & Sun, Q. (2009). Impact of baseline ECG collection on the planning, analysis and interpretation of 'thorough' QT trials. *Pharmaceutical Statistics*, *8*, 113−124.

Sheridan, C. (2010). Fresh from the biologic pipeline—2009. *Nature Biotechnology*, *28*, 307−310.

Simon, R., Ni, Q., Willems, R., Hartley, J. W., Daum, D. R., Lang, D., Ward, K., & Gill, J. (2003). Comparison of impedance minute ventilation and direct measured minute ventilation in a rate adaptive pacemaker. *Pacing & Clinical Electrophysiology*, *26*, 2127−2133.

U.S. FOOD AND DRUG ADMINISTRATION (2001). Guidance for Industry: Safety Pharmacology Studies for Human Pharmaceuticals (S7A). http://www.fda.gov/cber/gdlns/ichs7a071201.htm

U.S. FOOD AND DRUG ADMINISTRATION (2005). Guidance for Industry: Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals (S7B). http://www.fda.gov/RegulatoryInformation/Guidances/ucm129121.htm