**Natasha Nikolaidis and Candice Kissinger,**
BASi In Vivo Sampling Group, 2701 Kent Avenue; West Lafayette, Indiana 47906 USA

# Nicotine-induced Changes in Core Body Temperature and Locomotor Activity in Conscious and Freely-moving Rats

*Monitoring pharmacodynamic parameters such as core body temperature can be a useful tool in pharmacology studies. Our goal was to examine the usefulness of core temperature and locomotor activity as pharmacodynamic indicators, using nicotine as a test substance. In this study, we used an automated platform to conduct programmed dosing, temperature monitoring, and locomotor activity monitoring without human presence in the study room. Four rats were implanted with a gastric catheter and a temperature probe which could be externalized for direct connection to a power source and monitoring system outside the cage. Animals were housed in an interactive caging system which monitored locomotor activity, and connected to an automated dosing system and temperature monitor. The rats were intragastrically dosed with nicotine daily for four days (0.1, 0.3, 0.6, and 1 mg/kg). To control for possible development of tolerance, each rat was dosed in a different order. Core temperature and activity were continuously monitored for one hour before and two hours after dosing. Intragastric nicotine increased body temperature at all doses, with 0.6 mg/kg resulting in the greatest increase. 0.6 mg/kg nicotine also resulted in the greatest locomotor activation. Our data indicate that core temperature and motor activity can be useful pharmacodynamic indicators, as well as general indicators of animal health.*

Monitoring pharmacodynamic parameters such as core body temperature can be a useful tool in pharmacology studies. Many drugs can affect core temperature. Drugs such as ethanol and nicotine act directly on central thermoregulatory areas (1,2), while other drugs affect mechanisms such as vascular tone (e.g., hydralazine) which in turn can affect temperature (3).

One issue that must be addressed when measuring body temperature is how to prevent the measurement technique from affecting the parameter being measured. Stress causes increased body temperature in all mammals, which can persist for up to an hour after the stressful stimulus (4, 5). Popular methods for drug administration include restraint and manual injection or gavage, while temperature is often measured with rectal probes. All of these procedures are stressful. In animal models of anxiety, insertion of a rectal probe is a standard procedure to induce hyperthermia, which may then be blocked by anxiolytic compounds (4).

Since body temperature is extremely sensitive to stress, experimental methods requiring animal handling or restraint are likely to compromise reliability of the data. Therefore, methods of drug administration and temperature measurement that minimize restraint and handling have significant advantages over traditional methods.

We have developed a new hard-wired temperature probe compatible with the Culex® Automated Pharmacology System. The temperature acquisition software records temperature to a tenth of a degree Celsius, and allows the user to program sample intervals as short as one second, providing continuous temperature monitoring over the course of an experiment. By using an Empis® automated dosing accessory to deliver programmed drug doses, we were able to conduct an entire experiment without touching the animal. We chose nicotine as our test drug, since its effects on both temperature and locomotor behavior have been well characterized.

## Experimental Methods

### *Temperature Probes*

The new BASi temperature probe consists of a small thermistor sensor head, attached to a twisted pair of flexible, insulated leads, terminating in pin connectors. These probes are easily implanted in various areas, and externalized at the back of the neck. Because the externalized pin connectors are short, the rat can either be placed directly on the Raturn®, or returned to a home cage for recovery.

### *Surgical Procedures*

Male Sprague-Dawley rats were implanted with gastric catheters and allowed to recover for one week. In a second surgery, temperature probes were implanted in the inguinal area. A small incision was made in the skin, and the underlying connective tissue and muscle were blunt dissected to access the inguinal cavity. The head of the temperature probe was placed in the inguinal cavity, and secured to the underlying muscle with a single suture. The probe was externalized at the back of the neck, and a second suture threaded through the leads to secure the probe to the skin at the exit point. The rats were then placed in a Culex APS and connected to the Empis dosing system and the VitalScan® temperature acquisition system (BASi Vetronics). The rats were allowed to recover for 24 hours before experiments began.

### *Dose-Ranging Experiment*

Temperature and activity data were collected continuously for a minimum of 24 hours to establish the presence of normal circadian temperature variations, and the rats were then given a sham dose of 0.5 mg/kg of water intragastrically. Body temperature and activity were monitored for a further 24 hours.

Each rat was dosed four times intragastrically with a bolus dose of nicotine (doses: 0.1, 0.3, 0.6, and 1.0 mg/kg). Nicotine doses were spaced 24 hours apart, to allow the previous dose to clear from the system. The rats were dosed at 3 PM each day, and TEND was used to keep catheters patent between doses. The rats were fasted for 6 hours before intragastric dosing.

Nicotine infusion was automatically started by the Empis after the fasting period. In order to control for development of tolerance to increasing doses, each rat was dosed in a different order. Body temperature and activity were continuously monitored for 18 hours after nicotine infusion.

To control for differences in baseline temperatures and activity levels among animals, all averages are expressed as change from baseline. Baseline temperature and activity were calculated as an average of the 10 minutes just before dosing.

## Results

All rats displayed normal circadian variation in both temperature and activity (**F1**). Temperature and activity were higher during the dark cycle. Sharp decreases and increases in temperature and activity were correlated with lights on/off in the vivarium. In general, we observed greater circadian variation when animals were housed in quiet areas of the vivarium with minimal hallway traffic or on the weekends when no humans were present at all (**F2**).

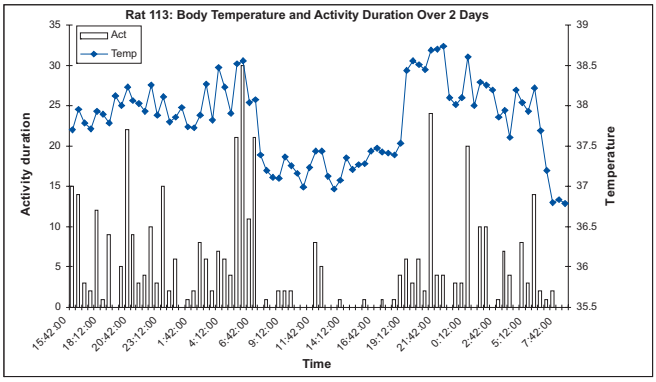
Intragastric nicotine increased body temperature at all doses in a dose-dependent manner, with the greatest increases occurring at the 0.6 mg/kg dose (**F3**). Locomotor activity followed a similar pattern. At 10 minutes after drug infusion, the 0.1 mg/kg nicotine dose caused the greatest locomotor activation (**F4**). For all other time points, a dose of 0.6 mg/kg resulted in the greatest activity.

## Discussion

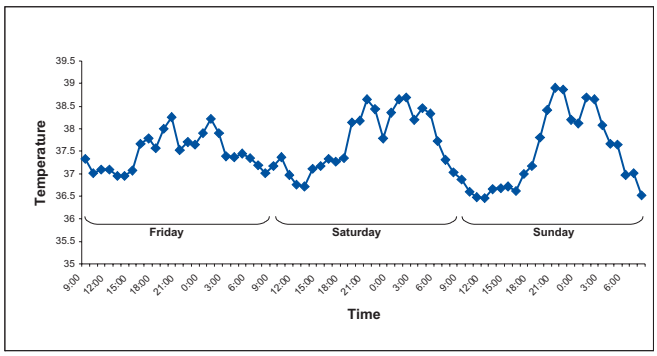
Body temperature is an easy pharmacodynamic parameter to measure, and changes in temperature may be an early indicator of CNS or endocrine response. In an animal catheterized for drug infusion and blood sampling, hard-wired temperature probes offer a simple method of data collection without animal handling.

Radio telemetry probes eliminate the need for repeated handling but suffer from two disadvantages. First, they are short-ranged and the receiver must be placed close to the animal (often under it), which will interfere with urine collection, and with movement of the Raturn. Second, interference from nearby electronics is a common problem and data may be lost. Hard-wired probes allow continuous data acquisition with no loss of data, and do not interfere with

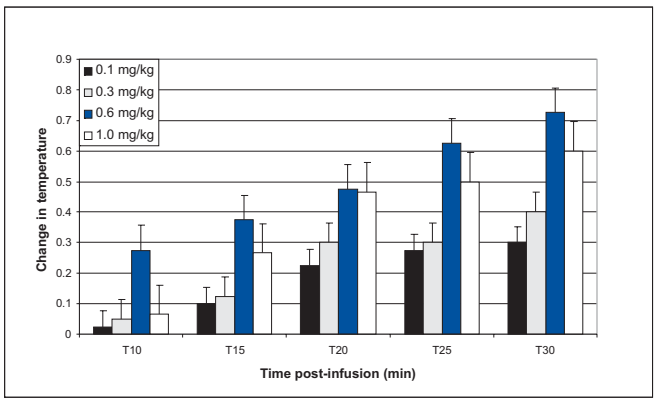
**F1**. Circadian rhythm patterns of temperature and activity for Rat 113.



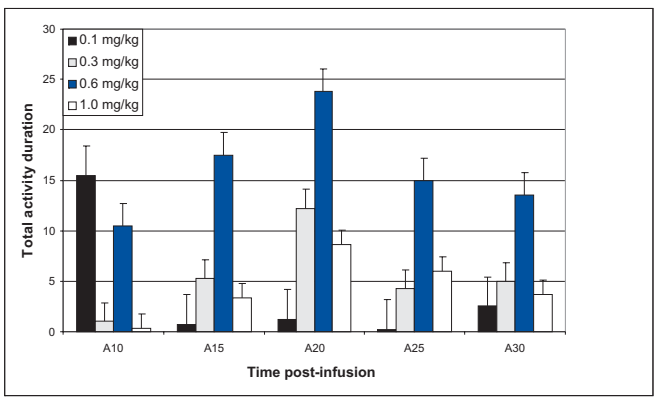

**F2**. Core body temperature variation in Rat 035 over three days.




**F3**. Average change in core temperature at four time points after nicotine dose.




**F4**. Average activity duration at four time points after nicotine dose.



measurement of other parameters such as urine flow or activity. This allows multiple subjects to be studied together, on the same cart, without interference.

This experiment provides evidence that body temperature can be useful as both a pharmacodynamic indicator, and as an overall indicator of animal health and well being. Animals in this study displayed a clear circadian rhythm which was affected by human activity. Drug infusion caused an immediate change in core temperature which correlated with increases in activity, and 0.6 mg/kg nicotine resulted in the greatest increases in both temperature and activity. At 10 minutes after nicotine infusion, 0.1 mg/kg nicotine resulted in the greatest activity. This was due to a brief and intense activity increase in a single rat. Activity levels for all the other animals remained close to zero at this time point.

Since temperature is so sensitive to stress and disturbance, quiet surroundings are critical to data collection. In the current study, we found that excessive traffic and noise outside study rooms caused flattening of the circadian rhythm, even though there were no obvious behavioral indicators to suggest that the animal was disturbed. When we moved the animal to a less noisy section of the vivarium, circadian rhythms patterns were more clear.

By using the Empis dosing accessory to deliver nicotine directly to the animal we also avoided the problem of human presence in the study room during dosing. Since Empis allowed us to program a time delay before administering nicotine, we were able to load the drug into the syringe, start Empis, and give the animal time to calm down before dosing. In this way, we were able to conduct an entire experiment from start to finish without human disturbance of the animals.

## References


1. *Crawshaw LI, Wallace H, Crabbe J., Ethanol, body temperature and thermoregulation, Clin Exp Pharmacol Physiol., (1998) Feb. 25(2):150-4.*
2. *Knox GV, Campbell C, Lomax P, The effects of acetylcholine and nicotine on unit activity in the hypothalamic thermoregulatory centers of the rat, Brain Res., (1973) Mar 15, 51:215-23.*
3. *Feller DJ, Crabbe JC, Effect of alcohols and other hypnotics in mice selected for differential sensitivity to hypothermic actions of ethanol, J Pharmacol. Exp. Ther. (1991) Mar 25 6(3):947-53.*
4. *Olivier B, Zethof T, Pattij T, van Boogaert M, van Oorschot R, Leahy C, Oosting R, Bouwknecht A, Veening J, van der Gugten J, and Groenink L., Stress-induced Hyperthermia and Anxiety: Pharmacological Validation, Eur. J. Pharmacol. (2003) Feb 28, 463(1-3):117-32.*
5. *Van der Heyden JA, Zethof TJ, Olivier B. Stress-induced hyperthermia in singly housed mice, Physiol Behav. (1997), Sep, 62(3):463-70.*