Implanted Telemetry Transmitters Alter the Noradrenergic Response in Vas Deferens from Mice

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Summary — Implantable telemetry devices are widely used in experimental animals. We investigated the potential for such implants to induce stress in mice. Changes in body weight and post-mortem responses of the vas deferens to noradrenaline (as a measure of sympathetic activation) were measured 28 days after transmitter implantation. We examined the influence of the anaesthetic used during implantation (pentobarbitone or fentanyl/fluanisone/midazolam), the strain (Balb/c and CBA) and the body weight at the time of implantation (small = 19–24g; large = 27–30g). A sham-implantation procedure did not significantly affect the responses to noradrenaline. When telemetry transmitters were implanted in small mice of both strains, there was a significant increase in the maximum response to noradrenaline compared to that obtained in tissues from large mice. The anaesthetic used during implantation did not affect the responses to noradrenaline obtained post mortem. Mice of both strains had a significant post-operative weight loss and this was maintained for the experimental period. The results show that implantation of telemetry transmitters has a significant impact in mice that weigh < 25g.

Key words: *mice, stress, telemetry, vas deferens.*

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Introduction

The use of telemetry is increasing in many areas of research. At present, an average of one or two papers are published each week, in which the methodology involves the use of implanted transmitters to measure one or more physiological parameters from conscious, unrestrained animals. One obvious reason for the growing popularity of this technology is that it allows simultaneous, continuous and accurate recording of a number of physiological variables without tethering, handling or restraint. It is generally considered that telemetry offers a humane approach to animal research, with the associated assumption that experiments can be conducted in the absence of stress on the animal. This is clearly a highly desirable aim; this paper addresses the question, "How well does telemetry achieve the Three Rs?".

Replacement requires the substitution of insentient material for conscious living higher animals. Since telemetry obviously involves the use of conscious, living higher animals, it cannot achieve replacement.

However, there are benefits of telemetry in terms of a *reduction* in the numbers of animals used to obtain information of a given amount and precision. The need to repeat experiments in a number of animals arises because of the variance among individual animals. With the use of telemetry, because the system is stable for months (and possibly even years) after implantation, animals can easily be used as their own controls. This reduces variance considerably, and there is a consequent reduction in the number of animals needed per treatment group. Furthermore, there may be no need for a control group of animals, so that the total number of animals used can be reduced even further.

The other aspect of reduction that should be considered is the relationship between the number of animals used and the quantity and precision of the data that are obtained. Telemetry provides an ability to continuously record a number of physiological variables, so that there is a significant increase in the amount of data that can be obtained from a given number of animals, compared to the use of conventional methods. Furthermore, there is no need for handling, restraint or externalised catheters and, in the absence of such potential stressors, the quality of the data obtained is improved.

In a study of the effects of ACTH on blood pressure in rats, Fraser *et al.* (1) obtained similar results when blood pressure was measured by tail-cuff and telemetry during 30-minute recording periods. However, when using telemetry, blood pressure measurement over a 4-hour testing session had greater statistical power than over 30-minute sessions. Furthermore, four hours of tail cuff recording would obviously be stressful for the animals. The authors concluded that telemetry gave greater statistical power to the study and required fewer animals.

Telemetry also makes a most effective contribution to *refinement*, decreasing the incidence or severity of inhumane procedures applied to those animals that still have to be used. For example, long-term measurement of blood pressure by the tail-cuff method would be stressful for animals but can be easily achieved in the absence of stress, using implanted telemetry devices. Earlier, more humane endpoints for toxicity tests can be set. The data acquired using telemetry can provide information about "normal" physiological processes and can be used to investigate the impact of housing, husbandry and/or other potentially stressful events. These data can be applied to identification of the most appropriate practices and the development of protocols with minimal distress and pain.

However, there is a real cost associated with the use of telemetry and careful consideration of the impact of all of the procedures involved in transmitter implantation and maintenance is required. Loss of body weight is generally accepted as being a valid indicator of the stress associated with experimental procedures and recovery of body weight is assumed to reflect recovery from the procedure. However, when considering recovery of body weight, it is important to note that unoperated animals would usually have continued to gain weight at a steady rate, so return to pre-operative weight does not necessarily imply total recovery.

Initially, general anaesthesia must be induced for surgical implantation of the transmitters. Anaesthesia *per se* is not without stress. Roughan & Flecknell (2) found that in rats (150–300g), general anaesthesia for 15 minutes, even in the absence of surgery, resulted in a small, but significant, weight loss of 2–7g. The general consensus is that it takes 2–4 days for an animal to recover from general anaesthesia and minor surgery. For example, after carotid artery catheterisation, completed in less than 15 minutes, recovery of body weight occurred by the fourth day after surgery (3).

The effect of more invasive surgery will obviously be more profound. A common approach for the implantation of transmitters includes laparotomy and placement of the transmitters in the peritoneal cavity. In one study in rats (4), all animals subjected to a 6cm laparotomy followed by gentle manipulation of the abdominal viscera for 5 minutes showed marked loss of body weight, which was not much reduced by administration of analgesics. Even after a smaller (3cm) laparotomy with gentle manipulation of abdominal viscera for only two minutes, rats had a mean loss of body weight of 11g, which was associated with a marked reduction in feeding and very obvious behavioural changes, e.g. abdominal/wound licking (2).

The potential impact of the procedure is increased when a telemetry transmitter is implanted. In our laboratory, implantation of transmitters in rats with body weights at surgery of 250–270g, resulted in weight losses of 15–20g in the two days after surgery (Figure 1) and disturbance in circadian rhythm (Figure 2). Animals had recovered their pre-operative body weight by day 4, at which time there was also a more well-defined circadian rhythm. By day 7, animals appeared to have fully recovered. In rats, the transmitter is likely to weigh considerably less than 10% of the body weight and is unlikely to be of major significance in the long term.

There is the common perception that mice are slower to recover from surgery than rats. Mice are usually assumed to need at least 4–7 days to regain normal growth rate after a simple laparotomy. There is a very real possibility of hypothermia during surgery and, when transmitters are being implanted, if the probe is not warmed to body temperature, it can act as a major heat sink and increase the risk of hypothermia. It is also important to recognise that a transmitter weighing about 3g in a 25g mouse represents a significant impact and there is the additional consideration of the volume of the device and the effect that it has on adjacent organs in the abdominal cavity.

The impact of telemetry implants in mice weighing 24–28g has been assessed by Baumans *et al.* (5). They showed that body weight was significantly reduced for the first four days after surgery and that it took 14 days for animals to regain their initial body weight. There was also a reduction in climbing, locomotion and eating and an increase in grooming and immobility. However, by the time body weight had recovered, the animals had also resumed normal behaviour.

As with rats, implantation of telemetry transmitters is associated with a disturbance of diurnal rhythm in mice. A typical trace obtained in our laboratory from a Balb/c mouse is shown in Figure 3. There was a considerable fall in body temperature associated with the implantation procedure, but

Figure 1: Weight changes (± SEM) in male Sprague Dawley rats (n = 5) after implantation of a telemetry transmitter

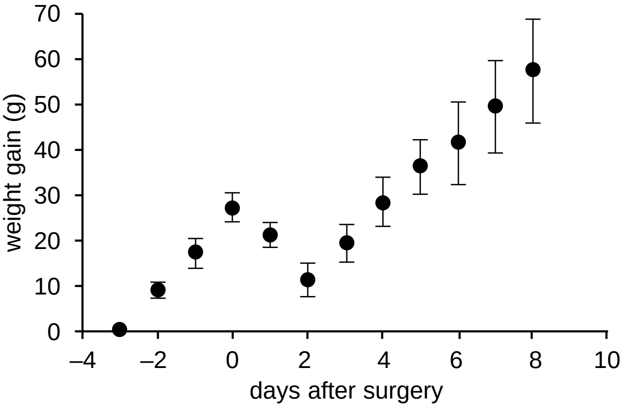
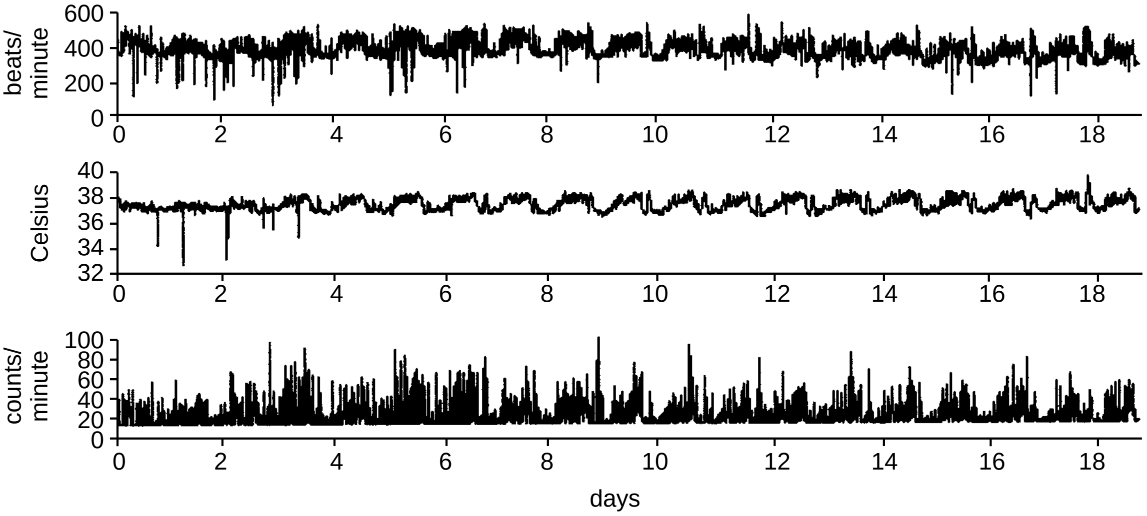


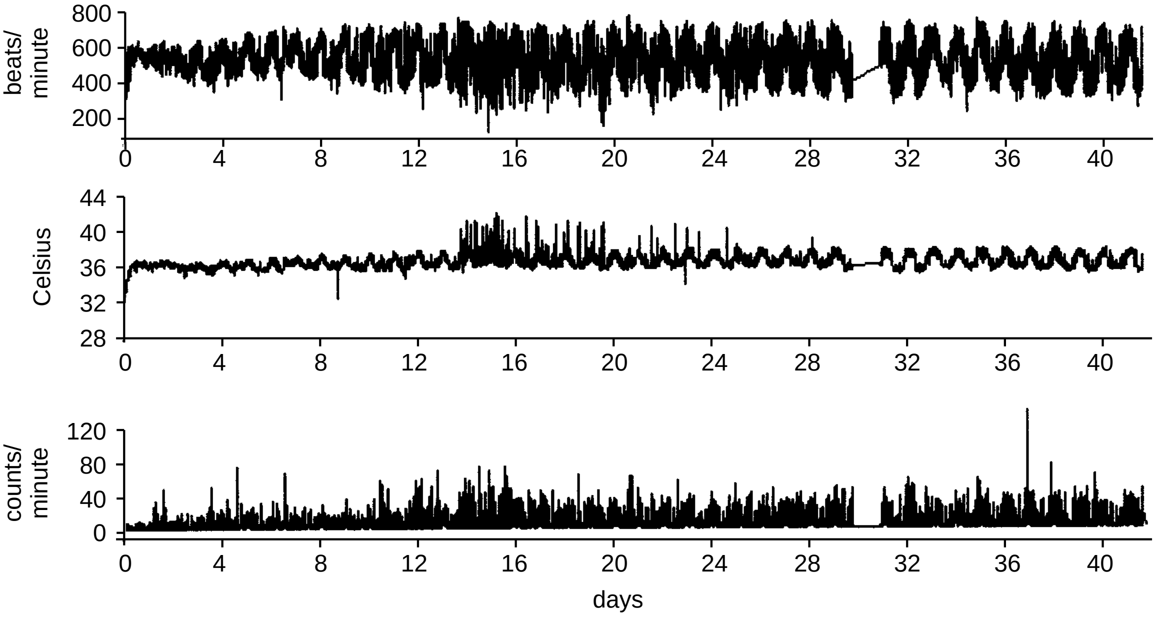
Figure 2: Typical recording of data from an implanted telemetry transmitter showing recovery of diurnal variation in heart rate (top trace), temperature (middle trace) and activity (bottom trace) in a male Sprague Dawley rat



this recovered over time. It generally takes up to 14 days for these animals to regain their diurnal rhythm, similar to the time found by Baumans *et al.* (5) for them to recover their pre-operative body weight.

In studies in our laboratory to assess the impact of common laboratory procedures and housing situations in rats and mice, results obtained using telemetry have been correlated with those from *in vitro* studies on tissues taken post mortem. These

Figure 3: Typical recording of data from an implanted telemetry transmitter showing recovery of diurnal variation in heart rate (top trace), temperature (middle trace) and activity (bottom trace) in a male Balb/c mouse



studies on isolated tissues are designed to detect changes that indicate activation of the sympathetic nervous system in response to imposed stressors. A consistent finding in mice has been that, in tissues from animals that have been stressed, the noradrenaline dose–response curve is moved to the left, and there is an increase in maximum response.

When we compared the mean of the dose–response curves of a group of control animals with implanted telemetry transmitters to that of a group of control animals with no implants, the curve was shifted to the left and there was an increase in the maximum response (6). That is, the implantation of a transmitter appeared to represent a stress. In these animals, the transmitter had been in place for 28 days, so the observed change was not simply an acute response to surgery. A series of experiments was designed to determine which factors contributed to the observed change in the dose–response relationship.

Materials and Methods

The experimental protocol was approved by the University of Sydney Animal Ethics Committee (K21/3-99/2/2908). Telemetry transmitters, weighing approximately 3.5g, identical in appearance to working transmitters (Data Sciences, TA10ETA-F20-L20), but without internal electronic circuitry, were implanted under anaesthesia with either pentobarbitone (50mg/kg, i.p.) or a mixture of fentanyl/fluanisone (Hypnorm) and midazolam (Hypnovel) and sterilised water in a ratio of 1:1:2 (0.07ml/10g, i.p.), to determine whether the general anaesthetic used had any influence. To evaluate the impact of the surgical procedure itself, a sham-operated group, which had a transmitter positioned for implanting, but which was then immediately removed, was included. Experiments were conducted in Balb/c and CBA mice to investigate whether strain was important in the effect, and mice of different weights were used (small = 19–24g; large = 27–30g). The transmitters were implanted (day 0) and allowed to remain in place for 28 days. Control animals were maintained for the same time. The mice were weighed twice weekly, and body weight changes were used as an index of stress. Animals were euthanised 28 days after implantation, and the vas deferens was removed for *in vitro* studies. Dose–response curves to noradrenaline were obtained, measuring the contraction as a percentage of the maximum response to a physiological solution in which potassium replaced sodium (KPSS). Analysis of variance was used to determine differences between groups of mice. Differences were considered to be significant when $p < 0.05$.

Results and Discussion

The changes in body weight are shown in Table 1. In the control animals of both strains, there was a small gain in body weight by day four and an approximately 12% increase in weight over 28 days. The sham-operated animals lost a significant amount of weight in the first few days after surgery, but had completely recovered by 28 days, and their body weights were not significantly different from controls. Thus, it could be concluded that the surgical procedure *per se* did not have a long-term effect on the animals. In contrast, for both strains of mice, neither the small nor the large implanted animals regained the weight that they had lost immediately after surgery, and all, even the small CBA mice, were still significantly lighter than the controls after 28 days.

The dose–response curves from the vas deferens of small Balb/c mice are shown in Figure 4. Sham-operated animals had dose–response curves that were not significantly different from control, confirming the body weight results, which indicated that surgery did not have long-term effects on the animals. However, in tissues from implanted animals, there was a shift

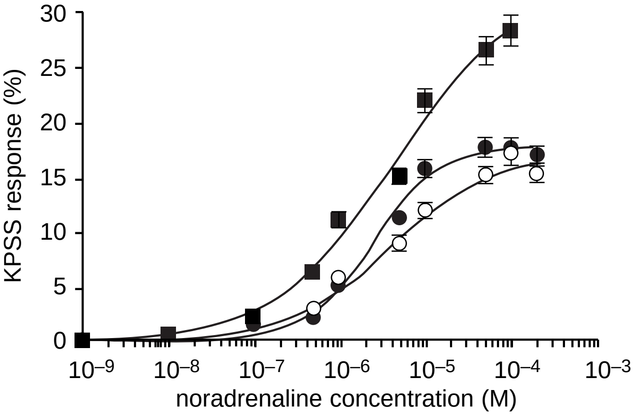
Table 1: Percentage change in body weights (means ± SEM) of Balb/c and CBA mice at 4 and 28 days after anaesthesia surgery for implantation of a telemetry transmitter

	Balb/c		CBA	
Group	Day 4	Day 28	Day 4	Day 28
Control (small)	1.9 ± 0.3	11.0 ± 1.2	4.7 ± 1.2	12.6 ± 1.4
Sham operation (small, p)	–4.3 ± 2.2	13.5 ± 1.1	—	—
Implant (small, p)	–13.7 ± 1.7	3.9 ± 4.1	—	—
Implant (small, h)	–9.8 ± 1.2	1.0 ± 2.1	–11.9 ± 2.4	7.1 ± 4.1
Implant (large, h)	–13.8 ± 3.3	–4.8 ± 3.1	–13.8 ± 1.2	–6.9 ± 0.6

Control animals had no procedures. Sham-operated animals had a transmitter positioned for implant and immediately removed.

h = anaesthetised with Hypnorm/midazolam; p = anaesthetised with pentobarbitone.

Figure 4: Dose–response curves for noradrenaline in the vas deferens of small Balb/c mice (anaesthetised with pentobarbitone for transmitter implantation)



Responses shown are means ± SEM; n = 6. Control animals (●) had no procedures. Sham-operated animals (○) had a transmitter positioned for implant and immediately removed. Responses in tissues from animals with implanted transmitters are shown by (■).

of the dose–response curve to the left and a highly significant increase in maximum response. Furthermore, since a similar shift to the left and a significant increase in maximum response also occurred in tissues from small Balb/c mice after transmitters had been implanted under fentanyl/fluanisone/midazolam (Figure 5), it was concluded that the anaesthetic used for implantation did not contribute to the altered sensitivity to noradrenaline. The observed changes were not peculiar to Balb/c mice, and similar effects were seen in tissues from small CBA mice. In contrast, although there was a small increase in maximum response to noradrenaline in tissues from large implanted mice (Figure 6), this was not statistically significant. Thus, it appears that the size of the animal is the major contributing factor to the apparent stress associated with long-term implantation of a telemetry transmitter.

This may become an even more significant problem with the use of transmitters to measure blood pressure in mice. These devices are larger and heavier (3.9g) than those used in our study. Furthermore, when the cannula is placed in the abdominal aorta, there is the added risk of compromising blood supply to the hind limbs. Mills *et al.* (7) implanted such transmitters in mice that weighed about 29g before surgery. They reported a 10% reduction in body weight the day after surgery and a further 2.5% in the next 2 days. It took approximately 2 weeks for the animals to regain their pre-surgery weights, and even after 30 days, they had not grown much from their pre-operative weight.

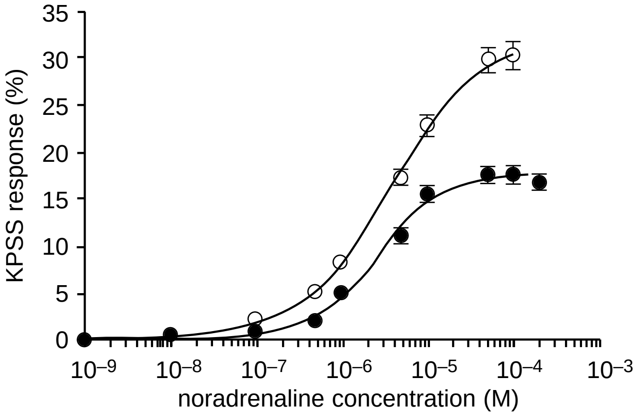
It has been suggested that subcutaneous implantion of these transmitters and cannulation of the carotid artery for blood pressure measurement may be more successful (8). With practise, the transmitters can be implanted within approximately 20 minutes, with recovery of food and water intake within 24 hours and body weight within 4 days (9). After this procedure, small female mice were still able to successfully mate, and there appeared to be no impact on any aspect of the pregnancy and postnatal development of the pups.

The expression of normal behaviour is an important part of an animal's basic physiological and ethological needs. Such behaviours include social interaction, and the potential impact of telemetry in this regard requires consideration. It is common practice to house animals in isolation while they recover from surgery and, since the technology in current use only allows recording from one animal per cage, they tend to be maintained in isolation for the duration of the experiment. Where there is evidence of stress associated with isolation, there is no reason why animals with implanted transmitters cannot be housed with an unoperated cage mate.

Conclusions

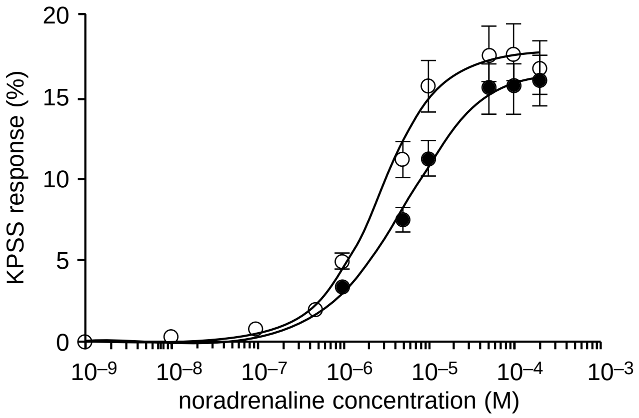
There is no doubt about the very positive benefit from many ongoing studies to determine optimum

Figure 5: Dose–response curves for noradrenaline in the vas deferens of small Balb/c mice (anaesthetised with fentanyl/fluanisone/midazolam for transmitter implantation)



Responses shown are means ± SEM; n = 6. Control animals (●) had no procedures. Responses in tissues from animals with implanted transmitters are shown by (○).

Figure 6: Dose–response curves for noradrenaline in the vas deferens of large Balb/c mice (anaesthetised with fentanyl/fluanisone/midazolam for transmitter implantation)



Responses shown are means ± SEM; n = 6. Control animals (●) had no procedures. Responses in tissues from animals with implanted transmitters are shown by (○).

husbandry practices and methods to minimise the impact of experimental procedures. There also does not appear to be any reason to challenge the claim that telemetry allows for a more humane approach to animal research. However, it is important that it is not assumed that telemetry will automatically deal with all animal welfare issues. There is still a need for careful consideration of each case and for determination of the most effective use of telemetry devices. This includes consideration of the potential impact of anaesthesia and major surgery for implantation and, especially in smaller animals, the impact of the device itself.

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