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Impact of an Implanted Intra-Abdominal Telemetry Transmitter on Fecal Excretion of Corticosterone and IgA in Adult Male F344 and BN Rats (*Rattus norvegicus*)

Niina Kemppinen, MS, PhD,[1] Anna Meller, DVM,[1] Otto Kalliokoski, MS, PhD,[2] Jann Hau, MD, PhD,[2] and Timo Nevalainen, DVM, MS, PhD, DipECLAM[3,4,*]

Telemetry is a widely used method for obtaining physiologic data from rats, but it is uncertain how distressing it is for the animals to live with an implanted transmitter for long periods. The present study aimed to assess this impact by analyzing 2 stress-sensitive biomarkers excreted in feces. Male Brown Norway (BN; *n* = 12) and Fisher 344 (F344; *n* = 12) rats were housed for 8 wk in IVCs and then for 8 wk in open top cages, in groups of 3, with one rat in each group carrying a transmitter. At 2-wk intervals, the rats were housed singly for 6 h (0600 to 1200), and voided fecal pellets were collected and frozen. Fecal glucocorticoids and fecal IgA from each rat were quantified and data subsequently analyzed using a repeated-measures mixed-model ANOVA. Both rat strain and transmitter carriage were found to significantly influence fecal corticosterone excretion. Overall, F344 rats excreted higher amounts of feces as compared with BN rats. In F344 rats with a transmitter the corticosterone values were 21% and in BN rats 47% higher than in controls, on average. Neither the rat strain nor an implanted transmitter seemed to have an impact on the amounts of fecal IgA excreted, but excretion increased significantly with age. In conclusion, in both rat strains, there was an increase in corticosterone excretion attributable to transmitter carriage, indicative of mild to moderate stress.

Abbreviations and Acronyms: BN, Brown Norway; F344, Fisher 344; IVC, individually ventilated cage

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Introduction

Telemetric methods enable a variety of biologic recordings from freely moving animals without interfering manipulations and without the presence of observers. As such, telemetric methods can be considered both refinement and reduction methods in the use of animals. Potential refinements and reductions for associated procedures and animal housing have been extensively reviewed in 2003 and 2004 by the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement.[1,2] One of these working groups encouraged studies in which resulting experimental variables can also be used to evaluate the welfare of the animals.[2] The group stated that it is widely recognized that telemetry can reduce stress to animals because it reduces or eliminates the requirement for external instrumentation, restraint, or tethering.[2] While acute stress is thus avoided when data are obtained through telemetry, the presence of chronic stress cannot be ruled out. Telemetry requires an invasive procedure in which a telemetric transmitter is implanted into the animal. Thereafter, the animal lives with a (potentially bulky) sensing and transmitting device within its body, potentially compromising its welfare. The recovery phase of transmittter implantation has been studied to some extent, but aspects of following after the recovery phase have gained little attention. Leon et al. (2004) studied the effects of transmitter size relative to the animal's body weight after intra-abdominal placement of a free-floating transmitter on body temperature, locomotor activity, growth, food and water intake, and diurnal rhythm in rats, but did not use postoperative analgesia. In that study, body weight did not decrease below starting levels, and food intake normalized by 2 d postimplantation. Diurnal temperature patterns and activity rhythms were detectable within the first week.[3] Greene et al. (2007) assessed mean arterial pressure, heart rate, body temperature, food consumption, and activity in rats for 2 wk following the abdominal implantation procedure and used a single injection of carprofen given the morning after sugery as analgesia. All parameters returned to presurgery levels by day 9, suggesting that these rats recovered from surgery in ~1 wk.[4]

Physiologic and psychologic stress are associated with an increase in circulating glucocorticoids. Ultimately, glucocorticoids and their metabolites are excreted through urine and feces.[5] Unlike serum sampling, samples of both urine and feces can be obtained noninvasively, although changes in glucocorticoid concentrations in the circulation become evident only after a delay. In rats, ~20% of the recovered corticosterone metabolites appear in urine with an average delay of 10 h, and 80% in feces with a delay of 16 h.[6–10] In a similar manner to corticosterone in the circulation, the metabolites in feces follow a diurnal rhythm; in contrast to serum corticosterone, the highest concentrations of

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[1]Laboratory Animal Centre, University of Helsinki, Helsinki, Finland; [2]Department of Experimental Medicine and University Hospital, University of Copenhagen, Copenhagen, Denmark; [3]Department of Basic Veterinary Sciences, University of Helsinki, Helsinki, Finland; and [4]Laboratory Animal Centre, University of Eastern Finland, Kuopio, Finland
**Corresponding author. Email: timo.nevalainen@uef.fi*

fecal corticosterone metabolites appear to be present in samples taken during the morning.[11,12]

Acute stress causes an upregulation of the immune system and leads to an increase in IgA.[13,14] If stress persists for a longer time, it may cause immunosuppression.[15] This can be assessed by quantifying secretory IgA in saliva or feces.[16,17] Fecal samples, which can be used for assessing both biomarkers, are easy to collect from rat cages without disturbing the animals, enabling long-term, longitudinal studies.[18]

The present study combined data from 2 of our previously published studies, focusing on the effects of cage furniture on cardiovascular parameters, locomotor activity, fecal corticosterone metabolites, and fecal IgA.[19,20] The aim of the present study was to combine and reanalyze these data to assess long-term welfare in rats carrying an abdominally implanted telemetry transmitter.

Materials and Methods

Ethical review. The study was done in the Laboratory Animal Centre, University of Helsinki, Helsinki, Finland. The protocol of the study was reviewed and approved by the Animal Ethics Committee of the University of Helsinki. The study complies fully with the EU Directive (2010/63/EU) on the protection of animals used for scientific purposes and the corresponding Finnish legislation.

Animals. A total of 12 Brown Norway (BN/RijHsd) and 12 Fischer 344 (F344/NHsd) male rats (Harlan, Horst, the Netherlands) were used in this study. The rats were 25 wk old and weighed 280 to 370 g (BN) or 350 to 460 g (F344) at the beginning of the study. The rats originated from barriers of a major commercial breeder but were housed in a conventional animal room. No systematic health monitoring was done, but the rats were found to have pinworms.

Animal housing and care. All rats were housed in the same room in polysulfone cages (Tecniplast, Buguggiate, Italy, 3 rats/cage). The cage type used was 1500U Eurostandard type IV S (44.5 × 33.5 × 21.0 cm; floor area 1,500 cm^2) with a solid bottom and with IVC double (first 8 wk) or open lids (following 8 wk). The cage floor was covered with 3.0 L of aspen chip bedding (size 4 × 4 × 1 mm, '4 HP,' Tapvei Oy, Kaavi, Finland). The cages were changed once weekly. The room temperature was 21.2 ± 0.3 °C (mean ± SD) and the relative humidity was 53.5% ± 7.7%. Artificial lighting with fluorescent tubes (light color: warm white) was provided from 0600 to 1800, and the light intensity in cages 1 m above the floor was 6 to 9 lx. Tap water was provided in polycarbonate bottles, changed once a week and refilled once in between. Irradiated (25 kGy) pelleted feed (2016 Global Rodent Maintenance, Harlan Teklad, Bicester, UK) was available to all rats ad libitum, except for the animals in the diet board group (see study design below), which were provided the food pellets inside holes drilled into a wooden board. A more thorough description is available elsewhere.[19]

Cage furniture and study design. Animals were housed in permanent groups of 3. The experiment used a crossover design with 2-wk periods where the animals rotated through the control condition and housing conditions with 3 furniture items: 1) cage without furniture (control condition); 2) cage equipped with a cross made of two intersected aspen boards (34.0 × 14.7 × 3.2 cm; 21.1 × 14.7 × 3.2 cm); 3) the same as condition 2, but holes (12 mm) were drilled into the boards that were loaded snugly with food pellets; rats had to gnaw wood to extricate food, no other food source was made available; and 4) cage provided with a rectangular aspen tube (20.0 × 12.0 × 12.0 cm [external dimensions]).

The specific item order is shown in Table 1.

Surgical procedure. Four rats of both strains were implanted with a radio telemetry transmitter (TA11PA-C40; Data Sciences International, St. Paul, MN). Anesthesia was induced with a mixture of fentanyl/fluanisone (Hypnorm, Janssen Pharmaceutica, Beerse, Belgium; 1 part), midazolam (Dormicum, Hoffmann–La Roche, Grenzach-Wyhlen, Germany; 1 part), and sterile water (2 parts) in a bolus of 0.15 to 0.20 mL/100 g SC. The ventral abdominal area was shaved and scrubbed with MediScrub, 1% triclosan solution (Medichem International, Sevenoaks, UK) and disinfected with chlorhexidine solution (Klorhexol 5 mg/mL, Leiras, Turku, Finland). Ocular lubricant (Viscotears, Novartis, Copenhagen, Denmark) was applied to both eyes. After a small incision along the ventral abdominal midline had been made, the transmitter presoaked in sterile physiologic saline was placed into the abdominal cavity and the catheter was put into the abdominal aorta. The transmitter was secured into the abdominal wall with 4-0 Ethicon Ethilon II sutures (Johnson & Johnson, St-Stevens-Woluwe, Belgium) and the incision was closed with 5-0 Ethicon polyglycolic acid suture (Johnson & Johnson). Postoperative pain alleviation was provided with 0.01 to 0.05 mg/kg SC buprenorphine (Temgesic; Schering-Plough Europe, Brussels, Belgium) twice a day and 5 mg/kg SC carprofen (Rimadyl; Vericore, Dundee, UK) once a day for at least 3 d. Parenteral fluids were given for 3 d. The pain medication for each rat was titrated against the individual response. The animals were allowed to recover for 10 d before recording was started.

Sampling. At the end of each 2-wk period, the rats were housed singly for 6 h (0600 to 1200) and all fecal pellets voided from each individual were collected and frozen (−18 °C). All samples were analyzed individually.

All 24 rats were weighed at 2-wk intervals starting when they were 26 wk old and ending at the age of 40 wk.

Fecal corticosterone and fecal IgA quantification. The extraction of both corticosterone and IgA was performed as described

Table 1. Illustration of the cage item allocation in each of the 2-wk rounds

Cage	Round 1	Round 2	Round 3	Round 4	Round 5	Round 6	Round 7	Round 8
1	PB	T	C	DB	PB	T	C	DB
2	DB	PB	T	C	DB	PB	T	C
3	C	DB	PB	T	C	DB	PB	T
4	T	C	DB	PB	T	C	DB	PB
5	PB	T	C	DB	PB	T	C	DB
6	DB	PB	T	C	DB	PB	T	C
7	C	DB	PB	T	C	DB	PB	T
8	T	C	DB	PB	T	C	DB	PB

Cage furniture codes: PB, plain board; DB, diet board; C, control; T, tunnel.

by Pihl and Hau.[17] The corticosterone ELISA was performed using a commercial corticosterone kit (DRG Diagnostics, Marburg, Germany) according to the manufacturer's instructions. The quantification of IgA was performed similarly to the assay also described by Pihl and Hau using reagents obtained from AbD Serotec (Kidlington, UK; purified rat IgA standard, PRP01), concentrations 0 to 1,000 ng/mL; coating antibody (mouse anti-rat IgA H chain, MCA191); and detection antibody (mouse anti-rat kappa/lambda L chain: HRP, MCA1296P, diluted 1:1,000).[17]

Data processing and statistical analysis. The data originated from 2 of our published studies.[13,20] The number of animals undergoing a procedure (transmitter implantation) was 8 and number of cage mates with no procedure (only collection of feces) was 16. With the crossover design, the theoretical number of samples is 24 (rats) × 8 (rounds) = 192. Samples for an IgA assay were collected during the last 5 rounds. Weight data consisted of 192 observations. The fecal corticosterone metabolite results, fecal IgA results, and weights of the individual rats were analyzed with repeated measures mixed-model ANOVAs on SPSS for Windows (version 14.0) using the strain, cage type, and cage item as factors and time as a repeated factor. Statistical significance was set at $P < 0.05$.

Results

Rat strain and transmitter carriage were found to be significant factors that impacted fecal corticosterone excretion. Both F344 and BN rats instrumented with transmitters showed higher excretion of fecal corticosterone (F(df1 = 1, df2 = 137) = 4.620, $P = 0.033$) than rats without transmitters; and F344 rats excreted more than did BN rats (F(df1 = 1, df2 = 137) = 6.026, $P = 0.015$). In F344 rats with transmitters the corticosterone concentrations were 21% and in BN rats they were 47% higher than in controls, on average (Figure 1). Fecal corticosterone levels did not display significant (F(df1 = 1, df2 = 137) = 3.253, $P = 0.73$) change during the 16-wk study (Figure 2). Both cage furniture and cage type were found to be nonsignificant ($P > 0.05$) factors for fecal corticosterone excretion.

Neither the rat strain nor transmitter carriage significantly altered the amount of fecal IgA excreted during 7 to 16 wk after transmitter implantation (Figure 3), but excretion in all rats increased with age (F(df1 = 1, df2 = 95) = 6.834, $P = 0.010$).

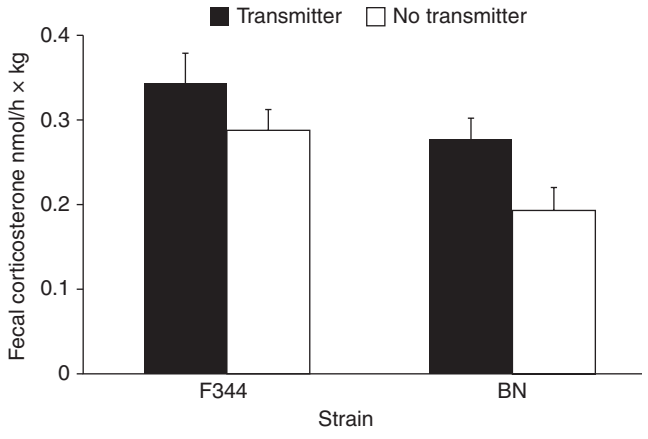

Figure 1. Fecal corticosterone excretion in 12 F344 and 12 BN male rats 2 to 32 wk after implantation of an abdominal telemetry transmitter. Results are expressed as means ± SEM. Both F344 and BN rats instrumented with a transmitter showed higher values for fecal corticosterone ($P = 0.033$) than did control rats. F344 rats excreted higher amounts as compared with BN rats ($P = 0.015$). Samples ($n = 168$, 55 samples from 8 transmitter rats and 113 samples from 16 rats without transmitter) were collected in 2-wk intervals.

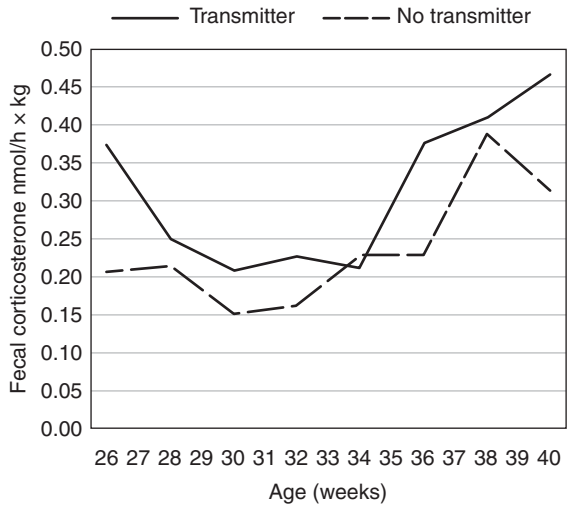

Figure 2. Means of fecal corticosterone values from 12 F344 and 12BN rats plotted against rats' age for both rat strains with and without transmitter. Samples ($n = 168$, 55 samples from 8 transmitter rats and 113 samples from 16 rats without transmitter) were collected in 2-wk intervals.

Body weight results displayed a significant (F(df1 = 1, df2 = 159) = 40,241, $P < 0.001$) transmitter carriage × strain interaction, which showed that both BN and F344 rats gained more weight with an implanted transmitter compared with their controls, but weight increase was larger for F344 rats than for BN rats (Figure 4).

Discussion

It is often assumed that implanted telemetry transmitters have no negative impact on the animals, but this is rarely the case.[2] Studies using intraperitoneally implanted transmitters usually consider rats to be fully recovered at ~10 d postoperatively.[3,4] The study described here evaluated the impact of transmitter carriage beyond that timepoint through the subsequent 16-wk period. Normally, it is during this period in which telemetry recordings are made. Hence, it is relevant to evaluate the stress and welfare of the animals during that recording period to assess whether chronic stress exists and if it might possibly exert confounding effects on research data.

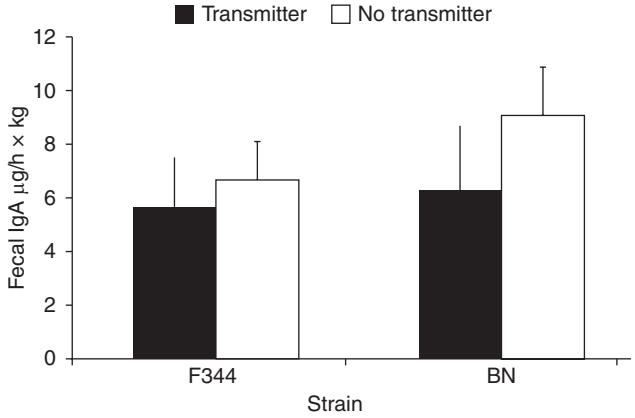

Figure 3. IgA excretion in 12 F344 and 12 BN male rats 14 to 32 wk after implantation of an abdominal telemetry transmitter. There were no statistically significant differences between the rat strains, but excretion increased over time ($P = 0.010$). Samples ($n = 125$, 43 samples from 8 transmitter rats and 82 samples from 16 rats without transmitter) were collected in 2-wk intervals.

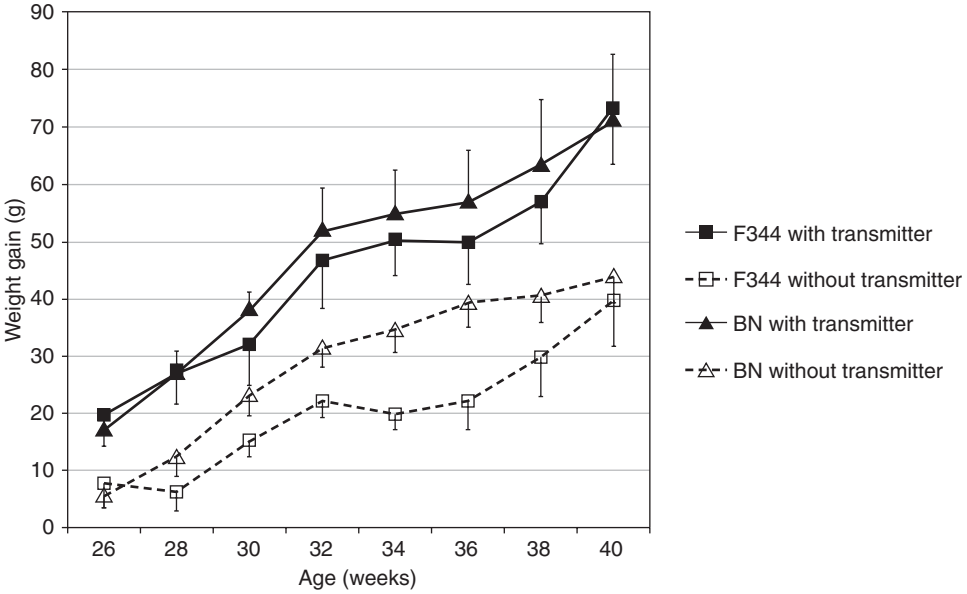

Figure 4. Weight gain of F344 and BN male rats 2 to 32 wk after implantation of an abdominal telemetry transmitter. Results are expressed as means ± SD. There was a significant ($P < 0.001$) transmitter carriage × strain interaction. Both F344 and BN rats gained more weight with an implanted transmitter compared to those rats without a transmitter during the 16-wk study period, but in F344 rats the difference was larger. Animal weights ($n = 192$, 64 from 8 rats carrying transmitter and 128 from 16 rats without transmitter) were recorded in 2-wk intervals.

Two inbred strains of male rats were chosen for this study, applying Festing's suggestion that several defined strains represent a species better than one single outbred stock.[21–23] At the same time, this study applied Fisher's principle whereby "a highly standardized experiment supplies direct information only in respect of the narrow range of conditions" while "deliberately varying in each case some of the conditions of the experiment, achieve a wider inductive basis for our conclusions, without in any degree impairing their precision."[24] Specifically, we used varying housing conditions such as 2 cage types and 4 different cage furniture types.

The main finding of the present study is that transmitter instrumentation leads to a significant long-term increase of fecal corticosterone levels (Figure 1), an increase that showed no significant change during the 16-wk study duration (Figure 2). Combining these findings with the design features of the study suggest that these results may be applicable to adult male rats as a group.

Guhad and Hau (1996) suggested that salivary IgA levels can be used to assess stress in rats.[16] There are no long-term studies on fecal IgA, but Erikson and coworkers showed significantly reduced concentrations during the 3 d after rats were transferred to metabolism cages.[25] The present study found no differences in fecal IgA attributable to the implanted transmitter during 7 to 16 wk after transmitter implantation (Figure 3). As a collateral finding we found increasing levels of fecal IgA over this 10-wk period. Increasing levels of fecal IgA also in the transmitter group do not support the notion of compromised welfare.

Weight gain is commonly used as an indicator of animal welfare.[26] This study showed that both F344 and BN rats with the transmitter gained more weight than those without, but in F344 rats the increase was larger (Figure 4). The weight gain may be due to stall of weight gain during recovery[3] and subsequent compensatory increase. We do not believe that the weight gain did not point to compromised welfare.

What are the items and procedures that could be causing the increased fecal corticosterone levels? The BVAAWF/FRAME/RSPCA/UFAW Joint Working Group recommendations state that "primary physical impact of a device on an individual animal will depend upon the relative size and mass of the device, its shape, the nature of the material from which it is made, its site and the method of attachment or insertion."[2]

The rats in this study weighed 280 to 370 g (BN) or 350 to 460 g (F344) and were 25 wk old at the beginning of the study. Mature, larger rats were chosen to decrease the relative size of the transmitter. The transmitter shape was cylindrical, and it weighed 9 g. The weight of the transmitter relative to the rats' body weights at the beginning of the study was 3.2% for BN rats and 2.6% for F344 rats. These numbers are small as compared with earlier studies on rat welfare. Somps (1994) used subcutaneous transmitters equal to 15% of a rat's body weight but saw no effect on growth, food and water intake, body temperature, or activity rhythms during a 14-d study.[27] Moran et al. (1998) used subcutaneous implants equal to 7.4% and 13.8% of a rat's weight for 90 d and observed no ill effects, except for an increase in adrenal weight with the larger implant.[28] The finding of increased adrenal weights is in line with our study, since adrenal hypertrophy has been connected to long-term increased glucocorticoid production.[29] It appears that a transmitter equal to 2% to 3% of a rat's weight could cause adrenal stimulation.

The BVAAWF/FRAME/RSPCA/UFAW Joint Working Group recommendations call for 'balancing' the placement of an implanted transmitter within the animal as much as possible, as a unilateral load can lead to device slippage and postural problems.[2] This study anchored the implant to the parietal peritoneum via suture inside the abdomen, roughly halfway between the forelegs and hindlegs. In this case, the implant site was as ventral as possible and balanced against the longitudinal or transverse axis, and therefore was unlikely to cause discomfort and pressure on adjacent tissues.

The BVAAWF/FRAME/RSPCA/UFAW Joint Working Group recommendations state that the ideal telemetry system would allow the animals to be housed in stable, compatible groups.[1] Cinque et al. (2018) showed that fecal corticosterone levels significantly increased after exposure to social and physical environmental enrichment in male and female rats at 72 h after exposure.[12] The present study used group housing, and the preestablished groups stayed the same throughout the

study except for individual housing of all rats for 6 h for fecal sampling every 2 wk.

Quantification of fecal excretion of corticosteroids has been shown to be a useful noninvasive measure of prior substantial stress (for example surgery), but it is not sufficiently sensitive to reveal minor stress or acute stress of short duration.[9] In the present study, the potential stress was chronic but there are no comparable studies on long-term effects of (mild) stress. It is remarkable that concentrations of fecal corticosterone increased consistently, regardless of strain or housing condition, making it highly unlikely to be a false positive result. Equally noteworthy is that the fecal corticosterone values were above control values in all but one time point during the 16-wk study (Figure 2). We conclude that instrumenting adult male rats with an intra-abdominal transmitter leads to mild to moderate chronic stress, which may complicate interpretation of telemetry studies.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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