A refined method for sequential blood sampling by tail incision in rats

Marc Fluttert, Sergiu Dalm and Melly S. Oitzl

Lab Anim 2000 34: 372
DOI: 10.1258/002367700780387714

A refined method for sequential blood sampling by tail incision in rats

Marc Fluttert, Sergiu Dalm & Melly S. Oitzl

Leiden/Amsterdam Centre for Drug Research, Division of Medical Pharmacology, PO Box 9503, 2300 RA Leiden, The Netherlands

Summary

Levels of endogenous or administered substances can be estimated by blood sampling. This allows an evaluation of the relationship between clinical signs, physiological parameters, pharmacological treatments and behaviour of the animal. We show that blood samples can be taken occasionally as well as sequentially by means of a small incision at the end of the rats' tails. Up to 300 µl of blood can be collected within 90 s. The advantages of this method are: (i) anaesthesia and surgery or restraint of the animal are not necessary; (ii) the procedure can be considered stress-free as indicated by the low, basal levels of the stress hormone corticosterone, even with frequent sequential blood sampling over 3 h; and (iii) it can be used for longitudinal studies allowing intra-individual comparisons over months and even years. Blood samples collected via an intravenous catheter and, at the same time, by our tail incision method resulted in comparable amounts of corticosterone. Moreover, we consider the tail incision method for rats to be 'animal-friendly' and a real alternative to other conventionally used blood sampling techniques.

Keywords Blood sampling; tail incision method; rat; corticosterone; stress environment; animal welfare

There is an increasing demand for techniques that really do allow easy, fast, reliable, repeated and 'animal-friendly' collection of blood. Methods for blood removal from laboratory animals have been reviewed by the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement (Morton *et al.* 1993). Most of these methods are unnecessarily stressful for the animal, simply because of the anaesthesia or restraint and the discomfort associated with a particular technique. For instance, restraining animals is one of the experimental techniques frequently used in stress research to elevate blood corticosterone levels, which reflect the activation of the stress system (McEwen & Sapolsky 1995). The main topic of our research concerns hormones involved in the regulation of the stress system and behaviour (de Kloet *et al.* 1998, 1999). In the past, we took blood samples from rats via an intravenously implanted catheter (jugular vein). This allowed us to measure the levels of hormones like corticosterone and ACTH in the blood under resting or activated states, e.g. in the home cage, in novel environments or related to emotional challenges (van Eekelen *et al.* 1991, 1995). Even if this proved to be an excellent blood sampling technique, we experienced several shortcomings

(1) When only one or two blood samples were required, rats had to undergo surgery and were thereafter housed singly to prevent them gnawing off each other's cannulae. Single housing of social animals like the rat for longer

Correspondence to: Dr M. S. Oitzl
E-mail: m.oitzl@lacdr.leidenuniv.nl

Accepted 31 May 2000

than a few days alters the stress-responsive system (Brain & Benton 1979).

(2) Blood samples could be collected safely for about 1–2 weeks, but there were always some failures. Thus, longitudinal experimental designs over months or years were not possible.

(3) Senescent rats are sensitive to surgery (Claassen 1994).

(4) Other methods described more recently as *refined* methods for blood sampling in rats, like tail-cuts (Liu *et al.* 1996: amputation of parts of the tail; Zeller *et al.* 1998: blood sampling from the sublingual vein; van Herck *et al.* 1991, 1998: orbital puncture) are not suitable for repeated blood collection in relation to behaviour, require anaesthesia and activate the corticosteroid stress system. For blood collection by decapitation at several time points, large groups of animals have to be used.

Consequently, we developed a procedure that was not hampered by any of these factors

During the last few years we have frequently used and have refined the technique of blood sampling by means of a small incision at the end of a rat's tail. Additionally, we have shown that levels of plasma corticosterone found in blood samples taken from a jugular vein cannula were comparable with those taken from a tail incision of the same animal. That the tail incision can be considered to be stress-free is expressed by the continuous low values of corticosterone from rats observed in their home cage. In contrast, simply placing a rat into a novel cage (a procedure, which is performed weekly in the animal house) increases corticosterone levels, indicating a stress response.

Materials and methods

Animals and experiments

Data of the present study are derived from male Wistar (200–250 g bodyweight; Charles River, The Netherlands). Animals were housed in pairs with free access to food (2122 SMR-A 10 mm, 0.9 mRAD; Hope Farms, Woerden, The Netherlands) and water and a 12 h light/dark cycle (lights on at 08:00 h) in temperature (21°C) and humidity (50–60%) controlled rooms of the animal house at the Sylvius Laboratory, University of Leiden, The Netherlands. They were housed singly the evening before the blood sampling. Sequential blood sampling over 3 h was used (time points are given in Fig 2) and the amount of corticosterone was estimated. To establish the effect of the environment, rats ($n=9$) were placed into a novel cage or into their home cage during sampling. Two blood samples were collected with an interval of 60 min from a separate group of rats housed in their home cage ($n=6$). Under isoflurane anaesthesia rats ($n=5$) were equipped with a cannula in the jugular vein (Steffens 1969) and blood samples were collected from both the jugular vein and the tail incision. About 10 min after the onset of anaesthesia, one blood sample was taken. Thereafter, 1 mg/kg corticosterone (in PEG 300) was injected subcutaneously and blood samples were collected. This was part of an experiment where we tested which concentration of exogenous corticosterone would result in plasma levels comparable with the ones induced by novelty. Furthermore, glucose concentration and haematocrit values were measured in the blood collected by tail incision (300 µl) in a separate group of rats ($n=5$; Wistar, 300 g bodyweight) at the Central Laboratory Animal Institute at the University of Utrecht, The Netherlands. All experiments were performed during the light period, between 08:00 and 12:00 h when basal resting corticosterone levels of rats are low.

Animal care procedures were conducted in accordance with the EC Council Directive of November 1986 (86/609/EEC). All experiments were approved by the Animal Experiment Committee of the University of Leiden, The Netherlands.

Determination of plasma corticosterone

Blood samples were centrifuged (3000 rpm for 10 min at 4°C). The plasma was stored at −20°C. Plasma corticosterone was determined using an antiserum raised in sheep against corticosterone-21-hemisuccinate bovine serum albumin (generous gift of Dr Th. Benraad, University of Nijmegen, The

Netherlands) as described previously (Veldhuis *et al.* 1982) using a 1:10 ether extraction to avoid interference of other plasma components. Detection limit was 0.1 µg/dl; intra-assay variation 8% and inter-assay variation was 10%.

Determination of other parameters in the blood

For glucose measurement, blood samples were centrifuged (3000 rpm for 10 min at 4°C), stored at –20°C and colorimetrically analysed (Vitallab200, Stam BV, Epe, The Netherlands). For estimation of the haematocrit (cells per volume %) blood was collected in EDTA coated tubes, stored at 4°C and analysed the same day in a blood cell counter (model K-1000, Sysmex, Ijsselstein, The Netherlands).

Blood sampling procedure

This procedure via the tail incision is depicted in Fig 1.

Handling the animals before blood sampling

If several blood samples are required, the experimenters handle the rats for 4–5 days before the collection of blood begins. During the daily 2 min of handling, the experimental situation should be mimicked and rehearsed: place the rat on a towel; fold the towel and create a hole; allow the animal to explore the towel and a somewhat limited space formed by hands (Fig 1A); stroke gently along the

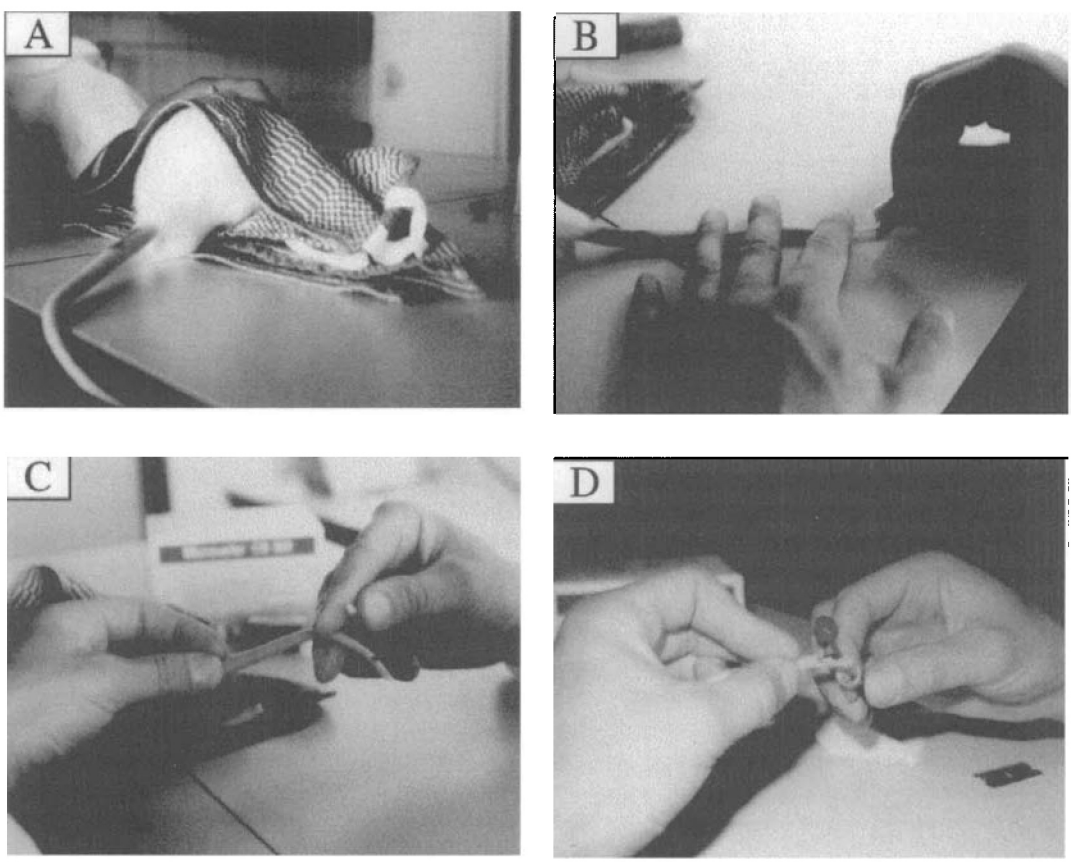

Fig 1 Blood collected by tail incision. (A) the rat is loosely held in a towel; (B) holding the rat's tail with one hand and performing the incision; (C) stroking the tail gently results in blood droplets at the incision; (D) collection of blood in a capillary

rat's tail; press the end of the tail with two fingers.

Blood sampling

The experimental procedure is most easily run with two persons: one pays attention to the animal, the other takes the blood samples. Blood samples can be taken from group- or singly-housed animals, in their home cage as well as before and after performing behavioural tasks. Interference with cage mates can be excluded by housing the animals singly overnight. This is also preferable, if more than one blood sample is collected within several hours.

Procedure

The rat is removed from its cage, placed on a towel on a table and held gently in place (Fig 1A). Rats keep quiet in this small and dark-surrounding. The rat should be restrained only if the experiment requires such a procedure. Figure 1B shows how to make the incision. Hold the end of the tail, fixed between two fingers, onto the table. Take a single edge razor blade and make a small, about 2 mm long, diagonal incision 15 mm from the end of the tail. Do it slowly. This incision will allow the collection of blood from the dorsal tail vein, which runs from the base to the tip of the tail. While making the incision, increase the pressure of the fingers on the tail above the incision. Generally, no avoidance response like tail flicking is observed. A drop of blood forms at the site of the incision. Take an EDTA-coated cup (Microvette CB 300, Sarstedt, Nümbrecht, Germany) or capillary tube suitable for blood collection. Figure 1C: Stroke gently with thumb, index and middle finger of your other hand from the base of the tail towards the incision. Again, a drop of blood is formed and can be collected. Avoid squeezing the tail because it is (i) painful for the animal and (ii) counteractive to the blood collection procedure as it induces vasoconstriction and may affect the quality of the sample. Repeat this procedure until the required amount of blood is collected (Fig 1D). Without further stimulation of the tail, bleeding stops and the rat can be placed back into its home cage. The collection of 300 µl of blood takes no more than 90 s from the opening of the lid of the cage until the returning of the rat to its home cage.

In one day, several blood samples can be collected from the same incision. Stroking over the incision gently with a hydrophilic gauze sponge dipped in saline re-opens it. Then, the tail has to be dried and the procedure of stroking the tail and collecting the blood droplets can be repeated. If longer time intervals (i.e. several days or months) occur between blood samples, new incisions have to be made, 1 to 3 mm away from the last incision, towards the base of the tail. Occasional or single blood samples can be taken without prior handling, since the whole blood collection procedure doesn't take long.

Results

Blood samples collected with an interval of 60 min contained low amounts of the steroid (µg/dl: time of incision: 1.31 ± 0.2; 60 min later: 1.15 ± 0.2). Animals were placed into their home cage after blood sampling. Placing an animal into a novel cage (i.e. clean cage with fresh bedding) resulted in elevated levels of corticosterone which were 8–10 times higher and returned to basal resting levels 120 min after novelty exposure (Fig 2; one way analysis of variance: $F(1,8)$ 21.841 $P = 0.002$). Corticosterone levels from animals in their home cage remained low (< 2 µg/dl). Even frequent sampling with an interval of 20–30 min only slightly elevated corticosterone levels. Comparable concentrations of corticosterone were found in blood from the jugular vein and the tail (Fig 3; Pearson correlation coefficient $r = 0.987$).

Blood glucose concentration was 6.96 ± 0.97 mmol/l (or 125.39 ± 17.4 mg/100 ml) and the haematocrit was $47.02 \pm 0.86\%$.

Discussion

Collecting blood by means of a tail incision is easy to learn and most of all, it is 'animal friendly'. Other methods reviewed by the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement (Morton *et al.* 1993)

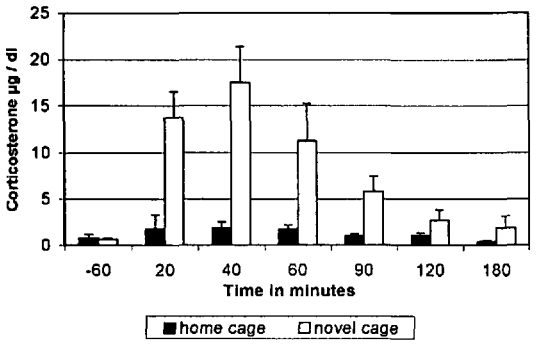

Fig 2 Effect of cage-environment on corticosterone levels. Sixty minutes after the tail incision rats were placed either into a novel cage where they remained during the following 180 min ($n = 5$; open bars) or taken out shortly and returned to their home cage ($n = 4$; black bars). Blood samples were taken 20, 40, 60, 90, 120 and 180 min thereafter. Note that exposure to a novel cage increases corticosterone levels over a long period of time

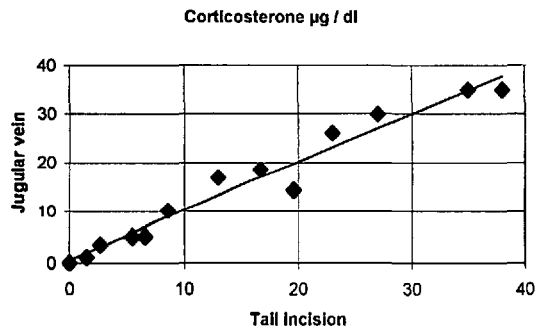

Fig 3 Comparable corticosterone levels are measured in blood collected from the jugular vein and tail incision ($r = 0.987$)

and still frequently used, incorporate a series of unnecessarily aversive conditions for the animals. For example, in order to insert a needle into the tail vein of the rat, it has first to be immobilized, e.g. in a restraining tube, and furthermore, the environment or the tail itself is heated up (Furuhama & Onodera 1983, Conybeare *et al.* 1988, Frank *et al.* 1991). Cutting off the tip of the tail, even under anaesthesia, leaves it prone to inflammation, is unnecessarily painful and requires intense post-surgery care (Verbaeys *et al.* 1995, Liu *et al.* 1996). Orbital puncture (van Herck *et al.* 1998), foot bleeding (Snitily *et al.* 1991), blood collection from the saphenous vein (Hem *et al.* 1998) or sublingual vein puncture (Zeller *et al.* 1998) cannot be used in behavioural studies. Both ether anaesthesia (orbital puncture) and restraint activate the stress system (van Herck *et al.* 1991, de Kloet *et al.* 1998) and therefore interfere with ongoing processes of learning and memory (de Kloet *et al.* 1999). Furthermore, all these methods exclude frequent blood sampling which causes much distress for an animal. As we have shown, even blood sampling via intravenous cannulae can be replaced by the tail incision method.

Blood glucose concentration and haematocrit values give a first indication for the quality of the blood collected by tail incision. The haematocrit (48%) is comparable with values reported by van Herck (1999)—haematocrit tail vein 49%, orbital puncture 50%. The concentration of plasma glucose in our study (125 mg/100 ml) is in the normal range (e.g. Besch & Chou 1971: glucose concentration was lowest in blood obtained by cardiac puncture—122 mg/100 ml, and highest in blood from ether-anaesthetized rats—165 mg/100 ml). Of course, studies comparing different blood sampling techniques are required to establish parameters regarding the quality of the collected blood.

The intense handling procedures preceding the blood sampling are most relevant in behavioural studies and if sequential blood samples are required. However, rats do not experience distress by taking repeated blood samples from the tail using this incision method, as proven by the low corticosterone levels. Moreover, with this method, occasional and small volume samples can be taken without (extensive) prior handling as it takes less than 30 s from opening the lid of the cage to the collection of 100 µl of blood. It can be easily combined with weighing the rat, and the method can be used in various rat strains, from youth to senescence (Gomez *et al.* 1996, Workel *et al.* 2000).

Taken together, the tail incision method provides a series of advantages over other blood sampling techniques. First, anaesthesia, surgery and restraint of the animal are not necessary. Second, the procedure can be considered stress-free as indicated by the low, basal levels of the stress hormone cortico-

sterone even with frequent sequential blood sampling over 3 h (Fig 2). Concentrations of corticosterone between 1 and 5 μg/dl are considered as low basal levels. Third, blood sampling by tail incision can be used for longitudinal studies allowing intra-individual comparisons over months and even years. In addition, the present study indicates the importance of the animals' home during and after sampling. Placing the animal in a novel cage, a simple procedure which happens weekly in the animal house and often before experiments begin, elevates the corticosterone levels. Restraint of the animal can boost corticosterone levels even more (Oitzl *et al.* 1995). In pharmacological studies one should be aware of the activation of the stress (and other physiological) systems as these might interfere with other measurements (O'Neill & Kaufmann 1990, Sarlis 1991, Morton *et al.* 1993).

The refined tail method for blood sampling in rats as well as a similar procedure for mice (Durschlag *et al.* 1996) is used regularly at our institute. Over recent years, both methods have been incorporated in the course given by the University of Leiden and Rotterdam. The Netherlands to acquire License Category C, i.e. persons responsible for directing animal experiments (see FELASA recommendations: Wilson *et al.* 1995). We consider blood sampling by tail incision to be a validated animal-friendly alternative to other conventionally used methods and techniques for collecting blood from laboratory rats.

Acknowledgments This study was supported by NWO grant 554-545. We thank Alexander Holzhäuer for arranging the photographs, Toon Hesp, Ron Timmermans (Central Laboratory Animal Institute, University of Utrecht) and Jaap Goekoop (Psychiatric Hospital Endegeest, Leiden) for analysing the haematocrit and blood glucose concentrations.

References

Besch EL, Chou BJ (1971) Physiological responses to blood collection methods in rats. *Proceedings of the Society for Experimental Biology and Medicine* **138**, 1019–21

Brain P, Benton D (1979) The interpretation of physiological correlates of differential housing in laboratory animals. *Life Sciences* **24**, 99–116

Claassen V (1994) Neglected factors in pharmacology and neuroscience research. In: *Techniques in the Behavioral and Neural Sciences*, Vol. 12 (Huston JP, ed). Amsterdam: Elsevier, pp 382–421

Conybeare G, Leslie GB, Angles K, Barrett RJ, Luke JS, Gask DR (1988) An improved simple technique for the collection of blood samples from rats and mice. *Laboratory Animals* **22**, 177–82

de Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M (1998) Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews* **19**, 269–301

de Kloet ER, Oitzl MS, Joëls M (1999) Stress and cognition: are corticosteroids good or bad guys? *Trends in Neuroscience* **22**, 422–6

Durschlag M, Wurbel H, Stauffacher M, Von Holst D (1996) Repeated blood collection on the laboratory mouse by tail incision—modification of an old technique. *Physiology & Behavior* **60**, 1565–8

Frank P, Schoenhart GL, Burton E (1991) A method for rapid and frequent blood collection from the rat tail vein. *Journal of Pharmacological Methods* **26**, 233–8

Furuhama K, Onodera TA (1983) A simple technique for repeated blood collection from the tail vein of the rat. *Journal of Toxicological Science* **8**, 161–3

Gómez F, Lahmame A, de Kloet ER, Amario A (1996) Hypothalamic-pituitary-adrenal response to chronic stress in five inbred rat strains: differential responses are mainly located at the adrenocortical level. *Neuroendocrinology* **63**, 327–37

Hem A, Smith AJ, Solberg P (1998) Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guineapig, ferret and mink. *Laboratory Animals* **32**, 364–8

Liu JY, Diaz TG, Vadgama JV, Henry JP (1996) Tail sectioning: a rapid and simple method for repeated blood sampling of the rat for corticosterone determination. *Laboratory Animal Science* **46**, 243–5

McEwen BS, Sapolsky RM (1995) Stress and cognitive function. *Current Opinion in Neurobiology* **5**, 205–16

Morton DB, Abbot D, Barclay R, Close BS, Ewbank R, Gask D, Heath M, Mattic S, Poole T, Seamer J, Southee J, Thompson A, Trussell B, West C, Jennings M (1993) Removal of blood from laboratory mammals and birds, First report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* **27**, 1–22

Oitzl MS, van Haarst AD, Sutanto W, de Kloet ER (1995) Corticosterone, brain mineralocorticoid receptors (MRs) and the activity of the hypothalamic-pituitary-adrenal (HPA) axis: the Lewis rat as an example of increased central MR capacity and a hyporesponsive HPA axis. *Psychoneuroendocrinology* **20**, 655–75

O'Neill PJ, Kaufmann LN (1990) Effects of indwelling arterial catheters or physical restraint on food consumption and growth patterns of rats: advantages of non-invasive blood pressure techniques. *Laboratory Animal Science* **40**, 641–2

Sarlis NJ (1991) Chronic blood sampling techniques in stress experiments in the rat—a mini review. *Animal Technology* **42**, 51–9

Snitily MU, Gentry MJ, Mellencamp MA, Preheim LC (1991) A simple method for collection of blood from the rat foot. *Laboratory Animal Science* **41**, 285–7

Steffens AB (1969) A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiology & Behavior* **4**, 833–6

van Eekelen JA, Rots NY, Sutanto W, de Kloet ER (1991) The effect of aging on stress responsiveness and central corticosteroid receptors in the Brown Norway rat. *Neurobiology of Aging* **13**, 159–70

van Eekelen JA, Oitzl MS, de Kloet ER (1995) Adrenocortical hyporesponsiveness and glucocorticoid feedback resistance in old male Brown Norway rats. *Journal of Gerontology: Biological Sciences* **50**, B83–9

van Herck H, Baumans V, de Boer SF, van der Gugten J, van Woerkom AB, Beynen AC (1991) Endocrine stress response in rats subjected to singular orbital puncture while under diethyl-ether anaesthesia. *Laboratory Animals* **25**, 325–9

van Herck H, Baumans V, Brandt CJWM, Hesp APM, Sturkenboom JH, van Lith HA, van Tintelen G, Beynen AC (1998) Orbital sinus blood sampling in rats as performed by different animal technicians: the influence of technique and expertise. *Laboratory Animals* **32**, 377–86

van Herck H (1999) *Orbital Puncture: A Non-Terminal Blood Sampling Technique in Rats* (Thesis). The Netherlands: University of Utrecht

Veldhuis HD, van Koppen C, van Ittersum R, de Kloet ER (1982) Specificity of adrenal steroid receptor system in rat hippocampus. *Endocrinology* **110**, 374–80

Verbaeys A, Ringoir S, van Maele G, Lameire N (1995) Influence of feeding, blood sampling method and type of anesthesia on renal function in the normal laboratory rat. *Urological Research* **22**, 377–82

Wilson MS, Berge E, Maess J, Mahouy G, Natoff I, Nevelainen T, van Zutphen LFM, Zanelly P (1995) FELASA recommendations on the education and training of persons working with laboratory animals: categories A and C. *Laboratory Animals* **29**, 121–31

Workel JO, Oitzl MS, Fluttert M, Lesscher H, de Kloet ER (2000) Persistent effects of maternal deprivation during life: hypothalamic-pituitary-adrenal axis activity and reactivity of Brown Norway rats. *Journal of Neuroendocrinology* (in press)

Zeller W, Weber H, Panoussis B, Bårge T, Bergmann R (1998) Refinement of blood sampling from the sublingual vein of rats. *Laboratory Animals* **32**, 369–76