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Cover Letter

Dear Ms/Mr,

We would be grateful if you would consider our manuscript entitled “Learning curve of cannulation of jugular and femoral vein in rats using Cumulative Summation Analysis” for publication in The Journal of Surgical Research. Our study investigates the learning curve required for competency and proficiency at blood vessel cannulation in rodents.

Vessel cannulation in rats, such as of the femoral and jugular vein, is a technically challenging procedure even for an experienced surgeon. In this study we analyse the results of 200 cannulations of the femoral and jugular vein in rats performed within one year by a single surgeon. The use of Cumulative Summation analysis allows calculating the required experience to most effectively cannulate the jugular and femoral vein, which is approximately 50 and 100 cases, respectively.

These results highlight the difficulty involved in performing such micro-surgical operations in rodents. The knowledge of the average time taken to cannulate these vessels allows experiments to be optimally planned, avoiding unexpected time over-runs. Furthermore, our results bring into the spotlight the need for training and direct supervision for the inexperienced researchers, as there is an ongoing need to reduce animal use in research through minimising adverse events.

To the authors’ knowledge this is the first study to report the learning curve for mastering the technique of catheterisation of the femoral and jugular vein in rats. We feel it will be of interest to readers of The Journal of Surgical Research, and particularly those using rodent microsurgery.

Yours faithfully

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Learning curve of cannulation of jugular and femoral vein in rats using

Cumulative Summation Analysis.

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Running title: A study on the learning curve of the catheterisation of the jugular and femoral vein in rats.

Subject category: Research

Authors' contributions

I.C., J.C., R.S. carried out the design and coordinated the study, participated in all of the experiments and prepared the manuscript. J.M., P.G., V.C., F.P., K.M., S.B. provided assistance in the design of the study, coordinated and carried out most of the experiments and participated in manuscript preparation. T.T., F.P., K.M. and S.B. approved the final version of the manuscript. All authors have read and approved the content of the manuscript.

Abstract

Background:

Intravascular access routes are widely used for administering agents or taking blood samples in rodents. Vessel cannulation in rats is a technically challenging procedure with a risk for significant complications. The use of Cumulative sum (CUSUM) analysis allows continuous monitoring of the performer's outcomes to evaluate the learning curve for a particular procedure. The aim of the present study was to assess a researcher's learning curve in the cannulation of the jugular and femoral vein in rats using CUSUM analysis.

Materials and Methods:

A single researcher performed two hundred micro-surgical operations between September 2012 and September 2013. The animals (male Wistar rats) were anaesthetised with isoflurane while the right jugular vein and the left femoral vein were catheterised. Prospective data were collected and analysed using CUSUM analysis. For the purposes of the study the rat population was divided in four groups based on the order of studies; group 1 represents the first 50 animals cannulated, group 2 the next batch of 50 animals and so forth.

Results:

The operating times required for cannulation of the jugular vein for groups 1, 2, 3 and 4 were 24.6 ± 4.8 min, 15.9 ± 2.5 min, 15.2 ± 3.2 min and 15.7 ± 3.3 min respectively. Group 1's operating time was significantly longer than all the other groups ($p < 0.001$ compared to all other groups). The operating times for groups 2, 3 and 4 did not differ significantly ($p > 0.05$).

The cannulation of the femoral vein required a mean of $32. \pm 5.3$ min for group 1, 24.9 ± 5.7 min group 2, 18.4 ± 4 min for group 3 and 17.2 ± 3.4 min for group 4. The

operating time of group 1 was significantly longer when compared to all groups ($p < 0.001$ for all groups). Group 2 also had a longer operating time than groups 3 and 4 ($p < 0.001$ compared to both groups). Groups 3 and 4 did not show any statistical significant difference when their operating time was compared ($p > 0.05$).

CUSUM analysis suggested that the number of cases required to achieve the required experience to most effectively cannulate the jugular and femoral vein is approximately 50 and 100 cases, respectively.

The adverse effects of the procedure included two unexpected deaths, both of which occurred in group 1 (0.5% in total).

Conclusions:

The authors' experience regarding the learning curve of the cannulation of the femoral and jugular vein in rats from 200 animals operated over a period of one year for the evaluation of the pharmacokinetic properties of drug candidates suggests significant experience is required to optimise the operating time required for the procedure.

Keywords: Rats, animals, operating time, jugular vein, femoral vein, catheterization/instrumentation, infusions, parenteral/instrumentation

Introduction

The concept of a “learning curve” was first described in industry in 1936 by T.P. Wright [1], who described a basic theory for costing the repetitive production of airplane assemblies. The concept has subsequently been used in many fields, with surgery, and especially heart surgery, being the driver for the introduction of the term in the medical literature [2, 3]. The experience acquired from performing a technical procedure differs among different individuals; and that the number of procedures completed alone is an inadequate marker of competency [4, 5]. Nevertheless, despite this variation, learning curves are often similar for many individuals for specific procedures, and knowledge of the learning curve for an individual for a specific procedure can help guide the training of subsequent practitioners.

The Canadian Association of General Surgeons in 2008 proposed four steps required to acquire new surgical skills [6]. Firstly, the surgeon must be or become knowledgeable about the condition for which the skill is to be applied—indications, complications, and technical and safety knowledge pertaining to equipment. Secondly, the surgeon must obtain familiarity with the technology and technique through observation of experienced operators. The third step is that the initial experience should be proctored, and the final fourth step occurs when the surgeon begins to practice the technique independently and an outcome assessment is undertaken to ensure that the quality is in line with accepted practice. Even under ideal conditions of training and supervision, there will always be a learning curve during the initial phases. A safeguarding system or technique should always be in place, for example in the form of a monitoring system that records the progress and results of the trainees, and identifies at early stages areas that need improvement.

Cumulative summation (CUSUM) is a statistical technique of sequential analysis that allows an observer to judge whether the variation observed in performance is acceptable or if the variation is outside that expected from random variation. CUSUM was developed during World War II in order to evaluate the efficiency of ammunition production lines. It has since been used in medicine for both assessing the competence of trainees and also in the assessment of doctors performing certain procedures in which they are already deemed competent [7-9].

CUSUM analysis is not only useful for medical procedures, but also for regulated in vivo procedures [10]. Vessel cannulation in both humans and especially in animals is a technically challenging procedure [11]. The purpose of this study is to use the experience obtained by a single surgeon at our Department over one year in the cannulation of the femoral and jugular vein in rats, as a model for the application of the cumulative sum analysis of a learning curve for these surgical techniques. The cannulation of these vessels was used in an animal model developed in-house in order to evaluate the pharmacokinetic properties (PK) of peptides developed as part of a drug discovery anti-obesity program. The use of animals for such PK experiments is standard practice and provides accurate and reliable results [12-17]. Optimal and timely vessel cannulation is important for this protocol; time is a critical component when performing PK experiments as there are limitations to time animals can remain under anaesthesia. Furthermore, failed experiments unnecessarily increase animal use. CUSUM analysis permits the early identification of successful or poor performance in vessel cannulation during PK experiments, thus allowing any necessary corrective measures to be taken in a timely fashion. To our knowledge, this is the first report regarding the learning curve of the cannulation of the femoral and jugular vein in rats using CUSUM analysis.

Materials and Methods

Animal studies

All animal procedures undertaken were approved by the British Home Office under the UK Animal (Scientific Procedures) Act 1986 (Project Licence 70/7236). Adult male Wistar rats (Harlan) (mean body weight: 558 g, range: 223-800g) were maintained in double-housed cages under controlled temperature (21-23°C) and light-dark cycles (12:12 hour light-dark schedule, lights on at 0700). Animals were allowed ad libitum access to water and ad libitum access to RM1 diet (Special Diet Services). To minimise non-specific stress effects, animals were regularly handled to allow acclimatisation.

Experiments

Animals were anaesthetised with isoflurane, a catheter inserted in the right jugular vein and a baseline sample taken. The catheters used were polyethylene tubing (internal diameter (ID) 0.46 mm, outside diameter (OD) 0.91 mm) (Instech Solomon) [18]. All cannula tubing required was prepared before the start of the procedure. The catheter was cut to 12 and 10 cm length for the jugular and the femoral vein respective. Catheter implantation was performed with the help of surgical loupes with a magnifying force of 2.5-3.5x. An infusion pump was used to administer agents. The desired flow rate and timing of the infusion were set (infusion time was 60 minutes at a flow rate of 0.3ml/h). Sampling of blood was performed through the jugular access. The constant intravenous infusion technique allowed the calculation of the pharmacokinetic properties of the peptide administered. Operated animals were divided in four groups (group 1-4) based on chronological order; group 1 represents

the first 50 animals, group 2 the next batch of 50 animals and so forth. The animals' body weights were not randomised between the four groups as this was not part of the study protocol. The operating time for cannulation of the jugular and femoral vein, and the unexpected events/deaths were recorded in a database. At the end of the experiment, animals were killed using a schedule 1 method under the Animals (Scientific Procedures) Act 1986 [19].

Anaesthesia and animal monitoring

Animals were anaesthetised with gas anaesthetic (isoflurane via cone mask at 2%) with an appropriate gas scavenging system. Induction was completed with oxygen flow at 0.8 to 1.5L/min and the isoflurane vaporizer to 3% to 5%, while in maintenance the face mask was connected to the Bain circuit and the oxygen flow adjusted to 2-3 L/min and the isoflurane vaporizer to 2 to 2.5%. Animals were regularly monitored throughout the procedure to ensure the correct level of anaesthesia was maintained. The physiological parameters of the animals were monitored with the use of a pulse oxymeter.

Jugular vein catheterisation

Anesthetised animals were placed on a heat mat, orientated with their back on the mat and their head facing the person performing the procedure, covered with a sterile drape and the forepaws slightly extended and taped to the heat mat. The skin from the right side of the neck extending from the jaw up to the right clavicle was lifted with angular forceps and a 2 cm vertical incision was performed with a surgical blade. Due to the tension, once the incision is performed the skin at the area separates and there is adequate exposure to the tissues underneath without the need for skin removal. The

platysma muscle was dissected and the right clavicle used as a guide to locate the point at which the jugular vein passes under the clavicle. Upwards dissection was performed in order to free the jugular from its medial and lateral branches. At the superior part of the incision, all branches of the jugular vein were identified and individually ligated. Blunt dissection was then used to clean the right jugular vein of fat and excess tissue until the vein was easily visible. The salivary gland was held out of the area of the vein using hemostats (Fine Science Tools, Heidelberg, Germany) and, with careful lifting of the vein, the area under the vein was dissected until a 2 cm section of vein was visible.

Three separate pieces of 4-0 Vicryl[®] suture were passed under the vein. One of these threads was moved to the rostral end while the other two pieces were moved caudally and a loose knot was prepared in each of them. The vein was put under slight tension by pulling the thread superiorly and with extreme caution a small incision (v-shaped cut) is made with the spring-scissors on the upper surface of the vein at about midway the distance between the superior and inferior ties. The pre-prepared tubing was inserted into the cut, and temporarily secured in place with tape. About 100µl of heparinised saline was infused through the catheter and the syringe was gently drawn back to allow approximately 100µl of blood to fill the tube and to ensure that the vein had been correctly cannulated. After confirmation of correct positioning of the catheter, the three loosely knotted threads were tightened to hold the cannula in place.

Femoral vein catheterisation

The left femoral vein was used as an access route for intravenous infusion of the peptide. The skin above the region was shaved and a horizontal 2 cm incision was made. As in the jugular area, once incised, the skin retracts allowing good

visualization of the structures underneath. The subcutaneous fat was dissected with blunt scissors and the left saphenous vein tied off and cut. The small lateral branches of the femoral vein were cauterised and by gentle traction of the saphenous vein, the femoral vein was lifted and dissected from its bed. The dissection extended from approximately 1 cm lateral to the point of insertion of the saphenous vein to as far medially as possible. The communicating branches of the femoral vein to the deep venous system were carefully ligated and cut or cauterised. The femoral artery and the femoral nerve were then dissected free from the femoral vein and held as far away as possible. The most lateral edge of the femoral vein was tied off with the help of a 4-0 Vicryl[®] ligature and the ligature was held with a small haemostat to provide lateral traction. Four individual ligatures of Vicryl[®] 4-0 were passed under the femoral vein and the three of them were used to secure the catheter in place once positioned. The fourth ligature, which should be the most medial, was used to control the proximal side of the femoral vein while inserting the catheter, and as the tie that occluded the femoral vein once the catheter was removed from the vein. The technique of inserting the catheter is the same as described previously in the jugular vein catheterisation, except that following the insertion of the catheter and the checking of its patency, the catheter was connected to the pump and the infusion started.

Sampling

There is a limit [$<10\%$ of total blood volume (2.56 ml) on any single occasion] to the amount of blood which can be safely extracted from small animals, which we did not exceed in our repeated sampling [20].

The Risk Adjusted Cumulative Sum (RA-CUSUM)

Time for cannulation was used to assess the learning curve in performing jugular and femoral intravenous lines in rats. Risk adjusted CUSUM is considered more accurate in evaluating the progress and the learning curve as it incorporates a risk associated with the observed surgery, which in this study was the body weight. Weight was found to be a significant factor determining the time required to complete the cases, and therefore used to risk-adjust the curve. The analysis in the present study was performed following the same methodology as reported elsewhere. Each case in the series was plotted on a chart from left to right on the horizontal axis. The line ascended for every case that was performed within certain time; and descended for every case that took longer to perform than the defined cut-off. The angle that the graph progressed was determined by the weight (3 groups) of each animal based on the Weibull survival model. Therefore, if there was a delay in a higher risk (heavier animal) case, the performance chart is not excessively penalized. There is higher drop in the graph for a lower risk case that is delayed.

The present study evaluated the learning curve for performing jugular and femoral lines in rats by one surgeon. Each case included in the RA-CUSUM analysis shows the operational time minus the expected time to complete the case, for the surgeon, in a chronological order.

Statistical analysis

All data are expressed as the mean value $\pm$ SEM. Comparisons of the operating time and the body weight between the groups were performed using one-way analysis of variation (ANOVA) with Bonferroni correction test. In all cases, values of $p < 0.05$ were considered statistically significant. The following programmes were used for

statistical analysis: (GraphPad Prism version 5.00 for Windows; GraphPad Software, San Diego, CA).

Results

Animal weight

Operated animals were allocated to four groups (group 1-4) based on chronological order, and to two sub-groups (jugular-femoral) based on the type of the vessel cannulated. The analysis of the body weights of the animals has shown that group 1 had the lowest body weight, with a mean of 419.5g (range: 223-706), and group 4 the highest body weight, with a mean of 614.2g (range: 533-878g) (figure 1). Groups 2 and 3 had a similar mean body weight (mean of 537.1g and 532.3 and range: 308-633 and 274-694 for groups 2 and 3 respectively). The body weight of group 1 was significantly lower than that of all other groups ($p<0.001$ when compared to all 3 groups). Groups 2 and 3 body weight did not differ significantly between them ($p<0.05$), while both groups differed significantly when compared to group 4 ($p<0.001$ for both groups).

Jugular Vein cannulation

The operating time required for cannulation of the jugular and the femoral vein is shown in figure 2. The first group of operated animals required a mean of 24.6min (range: 17-39min) for the cannulation of the jugular vein (figure 3), group 2, required 15.9min (range: 11-23min), group 3 15.2min (range: 8-22min), and group 4 15.7min (range: 7.9-22min). The operating time required for group 1 time was significantly

longer than that for all the other groups ($p<0.001$ for all groups). Operating time for groups 2, 3 and 4 did not differ significantly between them ($p>0.05$).

Femoral vein cannulation

The cannulation of the femoral vein required a mean operating time of 32.6min for group 1 (range: 23-48min), 24.9min for group 2 animals (range: 11-37min), 18.4min for group 3 (range: 8-26min) and 17.2min for group 4 (range: 8.8-23min) (figure 3). The operating time for group 1 was significantly longer than that for all other groups ($p<0.001$ for all groups). The operating time for group 2 was significantly longer than that for groups 3 and 4 ($p<0.001$ for both groups). The operating times for groups 3 and 4 were not significantly different ($p>0.05$).

RA-CUSUM Analysis for jugular vein cannulation

The observed and expected rate for performing the jugular lines within more than 18 minutes (mean time to complete procedure) was 68.8%, 27.9% and 21.7% for animals weighing less than 400 (group 1), between 400 and 599 (Group 2), and more than 600 (Group 3) grams, respectively. Therefore the RA-CUSUM plot for group 1 animals increased by 0.688 if the procedure was completed within 18 minutes, or decreased by 0.322 if not. It increased by 0.271 or decreased by 0.721 for group 2 animals, and increased by 0.217 or decreased by 0.783 for group 3. The learning curve for any complications is shown in Figure 4. The 48th case was when the learning point was reached, and the time taken to perform the procedure subsequently decreased.

RA-CUSUM Analysis for femoral vein cannulation

The observed and expected rate for performing the femoral vessel cannulation within more than 23 minutes (mean time to complete procedure) was 84.4%, 40.2% and 23.9% for animals weighting less than 400 (group 1), between 400 and 599 (Group 2), and more than 600 (Group 3) grams, respectively. Therefore the RA-CUSUM plot for group 1 animals increased by 0.844 if the case was completed within 23 minutes or decreased by 0.166 if not. It increased by 0.402 or decreased by 0.598 for group 2 animals, and increased by 0.239 or decreased by 0.761 for group 3. The learning curve for any complications is shown in Figure 5. The 96th was when the learning point was reached, and the time taken to perform the procedure subsequently decreased.

Adverse effects/Unexpected deaths

The adverse effects of the procedure included two unexpected deaths, both of which occurred in the first group of operated animals. No other unexpected deaths occurred in the rest of the cohort. Accidental dislodgements of the catheters did not occur, and the sample volume procured was adequate in all experiments. Occasional blockage of the jugular vein catheter due to repeated sampling was easily resolved by flushing with saline and minor re-adjustments of the catheter.

Discussion

This study describes a cumulative sum analysis of the learning curve of one surgeon for cannulating the femoral and jugular vein in rats. To the authors' knowledge, this is the first study to assess this learning curve. The RA-CUSUM analysis performed in this study shows that a significant number of cases are required in order to improve

the surgical technique and consequently the time required to complete these procedures. The operating time required for cannulation of the jugular vein ranged between 17 to 39 minutes at the beginning of the experience, and plateaued following approximately 50 procedures at about 15 minutes. Femoral vein cannulation took more time during the early stages (23-48min), and plateaued after 96 procedures at 23 minutes.

An interesting finding of this study is that the weight of the animals correlates with the operating time required to cannulate the vessels. There was a 2-to 3-fold decrease in the probability that the vessel cannulation would occur within the expected time as the weight increased from 400 g, to animals weighing 400-600 g and to more than 600 g. This may be related to the time required to dissect the subcutaneous fat and to clearing the vessel from the surrounding tissues. An increased operating time due to the a higher weight of the patient has been previously reported in human surgeries, especially in bariatric procedures [21].

This procedure should only be used when there is a specific need for a surgically implanted cannula, as this involves significantly more equipment and greater expertise than techniques such as tail bleeding, and puts the animals at greater risk than non-surgical methods. Indications include the need for a secure and reliable intravascular access to deliver drugs in the circulation, the requirement for a large number of samples to be taken, and the need to minimise the stress of the animal, as in the case of pharmacokinetic and drug metabolism studies [22].

Vessel cannulation in animals has been the cornerstone for performing PK experiments and is the standard practice in drug discovery as it has been shown to provide accurate and reliable results [12-17]. PK experiments involve administering the drug compound and taking blood samples in order to assess the biological effect.

One of the basic concepts underlying such *in vivo* rodent models is that there have to be two different intravenous (i.v.) routes; the first for infusing the drug and the second for blood sampling [23, 24]. In this study we evaluated the learning curve for cannulating the femoral and the external jugular vein, two of the most commonly used i.v. accesses (other including the saphenous vein and the carotid artery) [12, 13, 17, 25].

The importance of a timely and consistent surgical technique of vessel cannulation in PK experiments comes from the fact that time is a critical factor in such experiments. In PK studies, emphasis is put not only on the drug's plasma concentration but also at the specific time-point of the experiment. Anaesthesia is known to influence the cardiovascular status during surgery and thus a significant variation in the cannulation time between surgeries may result in differences in the plasma concentrations of the drug due to altered haemodynamic parameters.

Two deaths occurred surgery during the early phase of our experience. The first was likely related to a cardiovascular event secondary to excessive bleeding while attempting to cannulate the femoral vein. The bleeding at the time of surgery was rapidly controlled with the use of a cautery pen. The second death occurred during cannulation of the jugular vein while adjusting the length of the catheter within the superior vena cava. This may have contributed to the commencement of cardiac arrhythmias. The authors used a pulse oxymeter in subsequent experiments, to monitor the heart rate and rhythm along with the oxygenation. The total mortality rate was 0.5% (2/200).

This study highlights how one surgeon's -performance, reflected by the operating time, changes with experience. When setting up an experimental protocol, a reduction of the operating time acquired with increasing experience should be anticipated, and

the protocol may be adjusted accordingly. The knowledge of the average time taken to cannulate these vessels allows experiments to be optimally planned, avoiding unexpected time over-runs. The results of this study may prove to be useful to researchers when designing an animal study involving the cannulation of the jugular or the femoral vein.

Furthermore, animal studies in research are well regulated and have specific end-points that are clearly defined in the animal. In situations where there is over-running in surgical procedures due to difficulties in vessel cannulation, the animal needs to be culled in a humane way. Such events result in an unnecessary increase in animal use. Accurate knowledge of the anticipated operating times can help reduce these events by allowing more time at the early stages of a series of studies.

The present study has a number of strengths. Firstly, all of the statistical techniques used to evaluate the learning curve of one researcher resulted in similar conclusions, reinforcing the result that the learning curve was reached at approximately 50 and 100 cases for jugular and femoral vein cannulation respectively. In addition, the CUSUM technique has the advantage of taking into consideration the experience gained from each case in a timely fashion. The risk adjustment further increases the accuracy of the curve by incorporating the risk that can influence your results into the curve. In this study this was shown to be the weight of the animals. Another strength is the size of the sample, which is sufficiently large to accurately demonstrate the point that the learning curve is achieved.

Technical skill level varies among researchers and may depend on the individual's prior experience, training, inherent ability, and surgical skills. This study was not designed to investigate these differences and this may represent a limitation, as all operations were performed by a single researcher who is a surgeon trained in

minimally invasive procedures. It is likely that non-surgeons may require more time to standardise their technique and achieve their learning curve and achieve similar outcomes. Training and direct supervision is highly recommended for the inexperienced, and practising in cadavers can decrease the number of unexpected events and improve surgical technique during surgery. Although there are currently no prospective studies to support the latter, there is some evidence advocating the beneficial nature of cadaveric training in the curriculum of surgical trainees, and this may also be true of animal surgery [26, 27]. Future studies can evaluate the data presented here by comparing operating times demonstrated by researchers of different backgrounds.

To the authors' knowledge this is the first study to report the learning curve for mastering the technique of catheterisation of the femoral and jugular vein in rats. It would be of interest for future studies to investigate the influence of experience and operating time on other parameters such as the systemic inflammatory response, which has been shown to influence certain aspects of animal's physiology [28, 29].

Conclusion

The number of cases required to achieve the required experience to enhance the performance of jugular and femoral vein cannulation is approximately 50 and 100 cases respectively. Further work is required to validate these results, to identify further factors which influence performance, and to investigate their applicability to other researchers.

Conflict of interest

The authors have no conflicts of interest.

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Figure 1: Pre-operative body weight of male Wistar rats operated on in a pharmacokinetic study. Group 1 represents the first 50 animals to be operated; group 2 represents animals 50-100, group 3 animals 100-150 and group 4 animals 150-200 (n=50 per group).. Comparisons between groups were performed by one-way analysis of variation (ANOVA) with Bonferroni correction test. In all cases, values of $p < 0.05$ were considered statistically significant. ***= $p < 0.001$

Figure 2: Operating time required for the cannulation of jugular and femoral vein in male Wistar rats during a pharmacokinetic experiment. (n=200). Animals allocated to 4 groups according to the chronological order of the operation (Group 1: 0-50, Group 2: 50-100, Group 3: 100-150 and Group 4: 150-200).

Figure 3: Operating time for catheterisation of the jugular vein and femoral vein in male Wistar rats in a pharmacokinetic study. Group 1 represents the first 50 animals to be operated on, group 2 represents animals 50-100, group 3 animals 100-150 and group 4 animals 150-200 (n=50 per group). The 4 groups are divided in 2 subgroups based on which named vein was cannulated; jugular or femoral. Comparisons between groups were performed by one-way analysis of variation (ANOVA) with Bonferroni correction test. Values shown are mean $\pm$ SEM. In all cases, values of $p < 0.05$ were considered statistically significant. ***= $p < 0.001$.

Figure 4: Risk adjusted cumulative sum (RA-CUSUM) of operating time required to cannulate the jugular vein in male Wistar rats during a pharmacokinetic experiment rats, plotted against case number (n=200).

Figure 5: Risk adjusted cumulative sum (RA-CUSUM) of operating time required to cannulate the femoral vein in male Wistar rats during a pharmacokinetic experiment rats, plotted against case number (n=200).