Comparison of and Habituation to Four Common Methods of Handling and Lifting of Rats with Cardiovascular Telemetry

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Summary
Daily routines in the animal house may influence the results and interpretation of experiments. Handling is one such routine since it is necessary to immobilize animals for even minor procedures. This study assesses the influence of four common handling and lifting methods on cardiovascular parameters (blood pressure, heart rate) and locomotor activity of Sprague-Dawley and Wistar rats. Seven rats were implanted with radio-telemetry transmitters. After a recovery period, they were housed in groups of three with two intact rats. Each instrumented rat was subjected to the four methods of handling and lifting (scruff, encircling, plastic cone, lifting and holding by the tail on the arm) and, the same method was repeated during three consecutive weekdays. The method was changed every second week in a rotational order. Handling increased cardiovascular parameters for about 30 min, these changes being statistically significant ($p < 0.05$) as compared to control conditions immediately before the procedure. With holding by the scruff, the response duration decreased significantly from day one to days two and three, indicative of habituation to this procedure. Rats did not habituate to the cone handling, nor to encircling or lifting and holding by the tail; with the restraint cone being apparently the most disturbing. In conclusion, we have found that there are measurable differences in the impact of various handling and lifting methods and the correct choice permits refinement (one of the "Three Rs" of animal usage) of the procedure. Cardiovascular telemetry appears to be a useful method when used for refining procedures on animals, such as handling and lifting.

Introduction

Only a few basic rat handling methods are in common use. The method to be used is chosen mostly based on tradition and personal preferences: experienced handlers seem to prefer manual techniques whereas inexperienced handlers resort to device-based methods. Surprisingly, there are few refinement studies which have compared the distress associated with common handling methods and devices, combined with habituation to handling. There are studies conducted for other purposes from which some results can be extracted, usually examining one type of handling. When rats are caught daily and handled by moving them from one hand to the other, a decrease of serum corticosterone and prolactin levels are seen in both after eight days, but only in corticosterone after 15 days (*Dobrakovova & Jurcovicova, 1984*). A more recent study showed that picking up a rat by the tail and

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gently holding it for one minute, repeated for three, seven or ten consecutive days did not result in any attenuation of serum corticosterone (*Gadek-Michalska & Bugajski, 2003*).

Handling as a procedure is a potential stressor, which clearly can influence the well-being of the animals, and perhaps the variance of normal physiological values and hence the results of experimental procedures (*Baumans et al., 1994; Clark et al., 1997; Gattermann & Weinandy, 1996/97; Poole, 1997*). For the purposes of refinement, we need to identify the least disturbing handling technique, and see whether habituation to handling occurs after a few handling episodes.

The person doing the actual handling of the animal is important to the procedure, to the welfare of the animal and to the outcome of the experiment (*Augustsson et al., 2002; National Research Council, 1996b*). When comparing responses to handling, exclusion of all external disturbances, and use of the same, experienced handler throughout are prerequisites for valid comparisons.

Radio-telemetry provides a means of obtaining cardiovascular measurements from awake and freely moving laboratory animals (*Brockway et al., 1991; Harkin et al., 2002*). Radio-telemetry can be considered as the technique of choice for detection of small differences with accuracy (*Kramer et al., 2001; Lange et al., 1991*). Such differences may well occur between handling techniques and habituation to them.

This study was designed to assess the influence of different handling methods on blood pressure (mean arterial, diastolic, and systolic pressure), heart rate and locomotor activity of Sprague-Dawley and Wistar rats, and to find out whether any habituation to these handlings occur over three consecutive days.

Materials and Methods

The study was carried out in two facilities: National Laboratory Animal Center, University of Kuopio, Finland and Laboratory Animal Centre, University of Oulu, Finland. The main environmental factors were similar in these facilities and the experimental procedures were carried out identically. The study protocol was reviewed and approved by the Animal Ethics Committee of the University of Kuopio.

Animals and housing

Altogether 21 male rats were used in this study. Nine rats were barrier-bred Wistar (HsdBrlHan: WIST, NLAC, University of Kuopio, Finland) stock, and 12 were barrier-bred Sprague-Dawley (Hsd: Sprague Dawley®SD®, Harlan Netherlands) stock. The Kuopio facility possesses WIST rats (three of which were implanted), and the Oulu facility houses SD rats (four implanted).

The rats were 11-12 weeks old at the time of implantation (290-310 g). Each of the seven implanted rats was housed in groups of three with two intact rats for the duration of the study in polycarbonate (55 cm x 35 cm x 20 cm) open-top solid bottom cages. The cage racks were kept in a cubicle room (WIST rats) or in an open animal room (SD rats). The animal room temperature was maintained at 20 ± 2 ºC, relative humidity 50.0± 10% with lights 12 h on and 12 h off (lights on at 7.00). The animals had free access to food (R36, Lactamin, Södertälje, Sweden) and tap water was available in bottles. Aspen chips (Tapvei, Kaavi, Finland) were used as bedding, three litres per cage, and cages were changed once a week during the experiments. Rectangular aspen tubes (length 20 cm, height 11 cm, and wall thickness 1.5 cm) were used as enrichment items. The tubes were placed in the cages one week before implantation and were changed to new tubes at every routine cage change on Fridays.

Experimental design

All rats were housed in groups of three throughout the study. A single rat was chosen on a random basis drawing blindly numbers identifying a rat from each cage to be implanted. The rats were implanted with TA11PA-C40 telemetry transmitters (Data Sciences International, St. Paul, Minnesota, USA) under anesthesia with a mixture of Hypnorm® (0.315 mg/ml fentanyl and 10 mg/ml fluanisone; Janssen Pharmaceutica, Belgium) and

Dormicum® (5 mg/ml midazolam; Roche, The Netherlands) (*Kramer, 2000*). The aorta was cannulated cranial to the bifurcation through a midline abdominal incision and the telemetry device was implanted. After the surgery, the rats were placed in individual cages and kept warm for one day and then returned to the home cage. The rats were given a single dose of 0.01 mg per 100 g SC of Temgesic® (0.3 mg/ml buprenorphine hydrochloride; Reckitt & Colman Products Ltd, Hull, UK) for post-operative analgesia. Animals were allowed to recover postoperatively for six to seven days prior to the experiments. Receivers (Model RPC-1, Data Sciences International, St. Paul, Minnesota, USA) were placed under the cages to start data recording.

After the recovery period, the animals were subjected to the four different handling methods, each of which was repeated on the second and third day at the same time. At both sites one experienced person carried out the procedure. Handling was always commenced at 14.00 hours on Tuesdays, Wednesdays and Thursdays. Throughout each handling day the animals were otherwise left undisturbed, and nobody entered the room during the recordings. The rest period between the rounds used was twelve days, and animals were exposed to the three day procedure in a rotational order.

At the beginning of each handling session, the cage was removed from the cage rack, and the cover of the cage was removed. Before grasping the rat, the investigator placed his/her hand into the cage to let the rats sniff the hand. Each instrumented rat was maintained in the designated handling procedure for 15 s with gloves (Tru-Touch™ Clear Medical Examination Gloves, MaxxiM Medical Inc., Florida, USA). After the handling, animals were returned immediately to their home cage and the cage was placed back on the rack. Handling consisted of four types of handling and restraint:

A. Scruff.

The scruff of the neck was carefully grasped between the thumb and forefinger (*Hornett et al., 1988; Laber-Laird & Swindle, 1996; Lawlor, 1997; Rand, 2001; Svendsen & Hau, 1994; Williams, 1976*). The method is illustrated in Figure 1.

B. Encircling

For lifting, index and middle fingers were placed down along the sides of the rat's head with thumb and ring finger under the forelegs. The other hand supported the tail and the lower body (*Fowler, 1994; Lawlor, 1997; Rand, 2001; Rasmussen & Ritskes-Hoitinga, 1999; Sharp & La Regina, 1998; Williams, 1976*). The method is illustrated in Figure 2.

C. Restraint cones (Rodent Restraint Cones, AH 52-9586, Harvard Apparatus® Massachusetts, USA). The rat was placed into a cone and the large end was closed (*Hau & Van Hoosier, 2003*). The method is illustrated in Figure 3.

D. Handling by the tail

The rat was lifted by its tail between the thumb and index finger by grasping the tail base and immediately placed on the arm of the investigator (*Fowler, 1994; Rand, 2001; Williams, 1976*). The method is illustrated in Figure 4.

Collection of data and analysis

Output from the telemetry transmitters was sampled by using telemetry hardware and software (Data Sciences International, St. Paul, Minnesota, USA). These data were saved to the hard disk of a computer and subsequently transferred to other computers for statistical analyses (STATISTICA 6.0, DELTA-MM Corp.).

All parameters were recorded every 5 min for 10 s and stored. The recordings were started right before the handling and continued for 24 hours. Prior to the handling session, the control recordings for 24 hours were stored. Systolic pressure (SP), diastolic pressure (DP), mean arterial pressure (MAP), and heart rate (HR) data were extracted from the blood pressure recording. Locomotor activity (LA) was obtained from the system by monitoring changes in the received signal strength, which are attributes to the movement of the animal (*Brockway, 1991*). For technical reasons, LA data was obtained only from the WIST rats.

Figure 1. Illustration of scruff method (A) used in the study.

Figure 2. Illustration of encircling method (B) used in the study.

The cardiovascular parameters and the LA are given as mean values ± SD (standard deviation) of response. Repeated measures ANOVA was used to assess the differences between the control situations, different handling methods and repeated handling. Their responses were analysed using post hoc comparisons with a Newman-Keuls test.

Figure 5 illustrates the principle of the data evaluation and shows (Figure 5 A) heart rate after the handling and during the control situation (one hour per day before and at the same time as the handling procedure). In this and in all other cases, the average was calculated for handling response and its duration. The handling response duration (Figure 5 B) resulted from the value after handling minus the mean for a one hour period after handling. The response duration began with the first measured value after the handling and lasted until the first value below the mean of the one hour period after handling. This point was named the turning point. Nonparametric statistics (Mann-Whitney U-test) was used to assess the differences between duration of responses of different handling methods and between the next two days. Differences at $p < 0.05$ were considered statistically significant.

Results

MAP and HR responses obtained immediately after the handling procedures and during the control period are shown in Figures 6-7. Handled rats responded with a marked and significant ($p < 0.05$) increase in MAP and HR compared to the control situations. During control conditions, the mean arterial pressure was 103 ± 7 mmHg and heart rate 329 ± 40 BPM. All handling and lifting methods of animals caused elevations in MAP by 15 ± 7 mmHg (increase by 15%) and in heart rate by 111 ± 26 BPM (increase by 34%) compared to the control situation.

It is worthwhile to mention that there were statistically significant ($p < 0.05$) differences in heart rate between stocks under the control condition. The

Figure 3. Illustration of restraint cone method (C) used in the study.

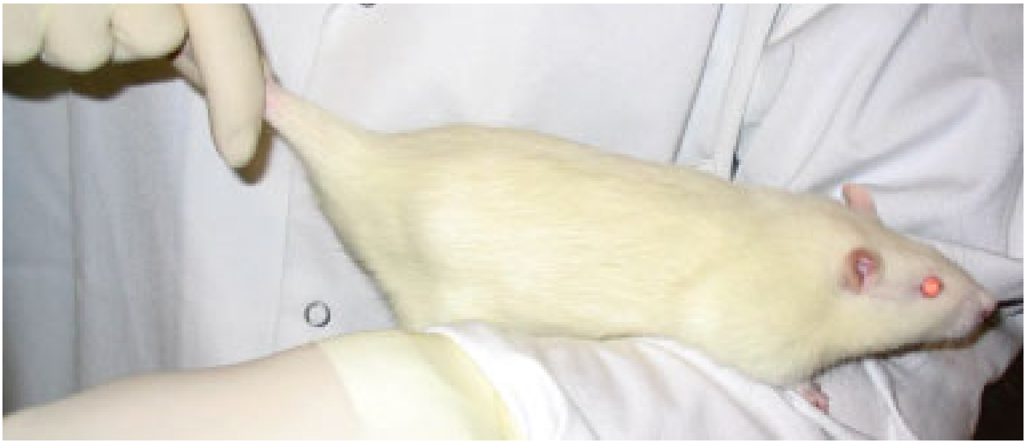

Figure 4. Illustration of holding by tail method (D) used in the study.

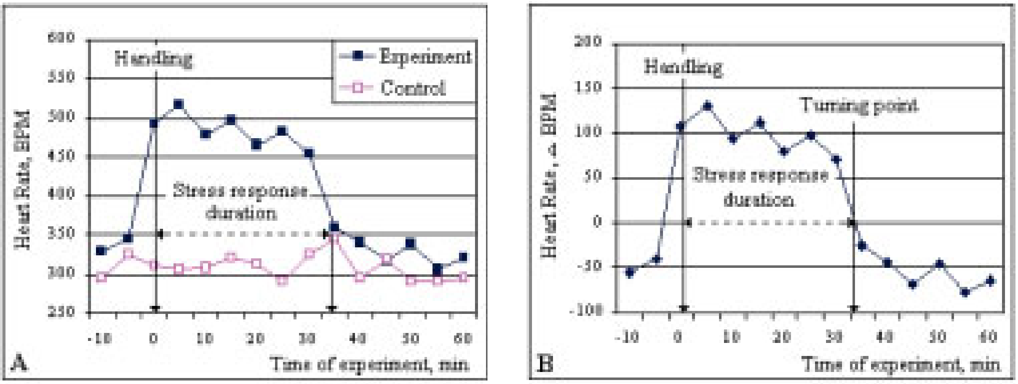

Figure 5. Calculation of the response duration. A. Typical effect of a stressor (handling) on heart rate in a rat and duration of effect (controls are used as the baseline). B. The heart rate after handling – data are expressed as change from the control for one hour period after handling. Turning point is defined as the first value below zero (for more details see text).

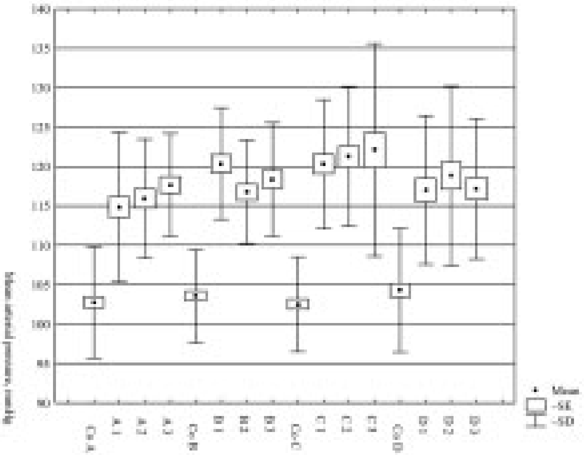

Figure 6. Mean arterial pressure (MAP) of seven rats during control situations and response to four types of handling (A-scruff, B-encircling, C-restraint cone, D-handling by the tail; Co A, Co B, Co C, Co D – control situation to respective handling methods obtained one day before the handling; A1-A3, B1-B3, C1-C3, D1-D3 - three day periods for handling, SE – Standard Error of Mean, SD – Standard Deviation). All handling methods resulted in a marked and significant ($p < 0.05$) increase in MAP in all three consecutive procedures compared to the controls.

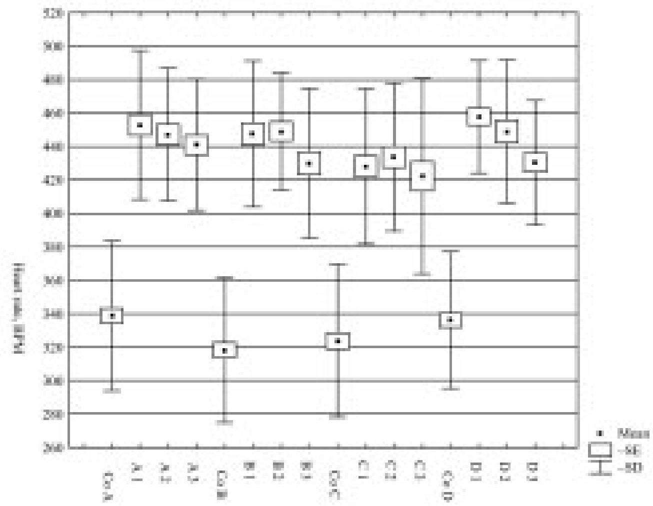

Figure 7. Heart rate (HR) of seven rats in control and response to four types of handling. (A-scruff, B-encircling, C-restraint cone, D-handling by the tail; Co A, Co B, Co C, Co D – control to respective handling methods obtained one day before the handling procedures; A1-A3, B1-B3, C1-C3, D1-D3 - three day periods for handling, SE – Standard Error of Mean, SD – Standard Deviation). All handling techniques resulted in a marked and significant ($p < 0.05$) increase in HR in all three consecutive procedures compared to the controls.

Table 1. Systolic and diastolic pressure during control (mmHg) and combined effect of all handlings used (ΔmmHg and %) in two stocks of rats. Results represent data from seven rats with a crossover design. Values are expressed as means + SD. Differences of responses (ΔmmHg) to the handling are statistically significant ($p < 0.05$).

Rats (n)	Systolic pressure		Diastolic pressure	
	Control (mmHg)	Response (ΔmmHg)	Control (mmHg)	Response (ΔmmHg)
SD (4)	125±14	+17 (14%)	87±6	+20 (23%)
WIST (3)	121±10	+11 (9%)	86±6	+14 (16%)
SD+WIST	123±12	+14 (11%)	87±6	+17 (20%)

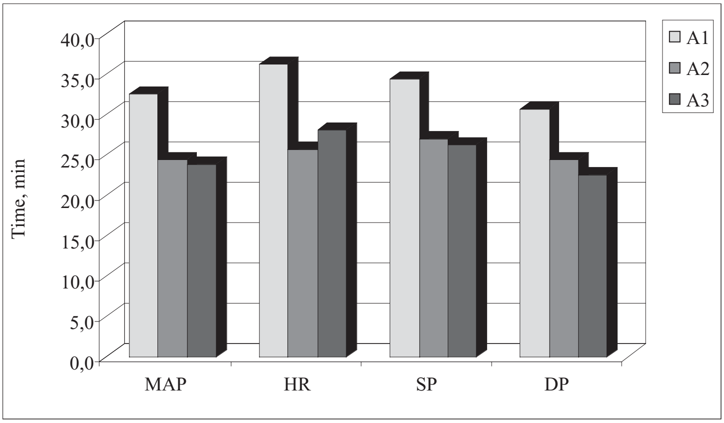

Figure 8. Duration of responses mean arterial pressure (MAP), heart rate (HR), systolic pressure (SP) and diastolic pressure (DP) from seven rats on first (A1), second (A2) and third (A3) day with scruff handling. The duration of the response was shorter in MAP, HR, SP and DP between the first and second, and between the first and third handling ($p < 0.05$).

heart rate of SD (318 ± 33 BPM) was lower than WIST (344 ± 47 BPM) rats during control period. The responses (ΔBPM) to handling exhibited the same pattern i.e. heart rate increased by 35% and 34% respectively. There were no differences ($p < 0.05$) between the stocks when comparing control MAP, but differences in their responses (ΔmmHg) to the handling were statistically significant ($p < 0.05$). MAP after pooled handlings increased by 18% in SD rats and by 12% in WIST rats. SP and DP showed the same responses as mean arterial pressure; these data are summarized in Table 1.

Handling of animals on three consecutive days did not seem to produce any lessening of the MAP, SP and DP responses. HR-values on the second and third days were lower than those detected on the first day (Figure 7) but the differences failed to reach statistical significance. Responses of MAP, SP and DP in the SD rats were statistically significantly ($p < 0.05$) higher to the restraint cone procedure when compared to the other methods.

Duration of the responses

The duration of cardiovascular responses to the handling ranged from 20 to 45 minutes. The average duration in both stocks to the different procedures during the three days for MAP ($p < 0.05$) and DP ($p < 0.05$) was shorter with the scruff method

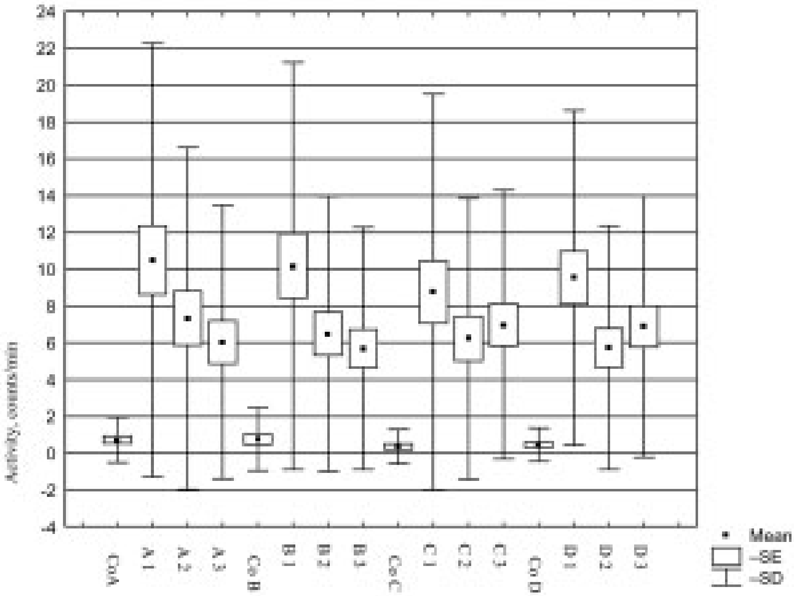

Figure 9. Locomotor activity of three Wistar rats during control and response to handling. (A-scruff, B-encircling, C-restraint cone, D-handling by the tail; Co A, Co B, Co C, Co D – control for different handling methods obtained one day before the handling procedures; A1-A3, B1-B3, C1-C3, D1-D3 - three day periods for handling, SE – Standard Error of Mean, SD – Standard Deviation). There were no significant differences in responses between any of the days.

compared to the cone. The duration of response was also shorter with scruff handling than occurred after lifting by the tail ($p < 0.05$). The duration of HR response was longer with the cone than with encircling ($p < 0.05$).

In most cases, the average duration to different handling procedures during three days for MAP, HR, SP and DP was significantly ($p < 0.05$) shorter with the scruff and encircling methods compared to the restraint cone.

The duration showed significant decreases on the next two days only for scruff handling (Figure 8). The duration of response was shorter in MAP, HR, SP and DP on the second and the third day as compared to the first day ($p < 0.05$). The areas under the curve (AUC) for MAP ($p < 0.05$) and DP ($p < 0.01$) were larger when rats were restrained with cones as compared to those with the scruff method.

Influence of handling on locomotor activity

Locomotor activity responses in WIST after the handling procedures and during control periods are given in Figure 9. In general, locomotor activity of the rats during control conditions was 0.7 ± 1 counts/min. After handling, locomotor activity increased to 14 ± 6 counts/min (range 6-30 counts/min).

Habituation to handling was evaluated between locomotor activities on consecutive days. Although locomotor activity values were lower during the next two days, there were no statistically significant ($p > 0.05$) differences between repeated handlings in WIST rats. However there were no statistically significant ($p > 0.05$) differences between different handling methods with respect to locomotor activity values.

Discussion

One of the most important aspects of the life of laboratory animals is how they relate with their human handlers and caregivers on whom they are totally dependent (*Poole, 1997*). Knowledge of the animal's normal response to handling is desirable, because individual animals can respond quite differently (*Grandin, 1997; National Research Council, 1996a*). When rodents are used for research it is of prime interest to reduce the discomfort that might be associated with even minor experimental procedures (*Hau & Van Hoosier, 2003; Lawlor, 1997*).

Depending on the study design, animal's responses to house routines may well influence the results of the experiments (*Augustsson et al., 2002*). Environmental disturbances have been shown to cause many physiological responses, including activation of the cardiovascular system (*Kramer, 2000; Lemaire & Mormede, 1995; Sgoifo et al., 1994*).

This study focuses on the influence of different handling methods on cardiovascular parameters and locomotor activity of rats. One of the advantages of this study is that we conducted measurements by using radio-telemetry, which is the most humane method for monitoring physiological parameters in freely moving animals (*Güler & Übeyli, 2002; Kramer et al., 2001*).

All handlings were done by one experienced person at each location. The handlers corresponded before the study in order to standardize the methods to be used. We consider this to be absolutely necessary; standardization to the smallest detail is essential if one wishes to combine data from two separate facilities. This study shows that results are in line between the facilities, which can be considered as a reflection of the good applicability of the outcome.

The results show that handling of the rats induced large and long lasting disturbances in the parameters monitored. This agrees with the findings of Harkin et al. (2002), who reported that handling provoked an increase in heart rate and body temperature for 40-50 min. Sharp et al. (2003) indicated that HR increased markedly and did not return to baseline until 60 min after handling procedures. Irvine et al. (1997) stated that after brief handling diastolic, and systolic blood pressure of Wistar/Kyoto rats and the spontaneously hypertensive rats had returned to normal after about 20 min. There is a paucity of studies in which habituation to common handling methods in rats have been compared. When habituation to handling has been studied, only one method of handling has been evaluated and most commonly serum hormones, such as corticosterone and prolactin, have been assayed (*Dobrakovova & Jurcovicova, 1984; Gadek-Michalska & Bugajski, 2003*). Collection of serum necessitates blood sampling, which can interfere with the interpretation of the results.

This study compared four different handling and lifting methods, and used non-invasive monitoring. The handling methods are inevitably a combination of procedures starting from opening of the door to the animal room until the departure of the last person in the task group. Another factor is the order in which the rats are handled. This study used a crossover design, which is more precise than a parallel design in outbred animals (*Festing et al., 2002*), with a rotational order such that each rat was exposed in every round to one of the methods. A rotational order is necessary if one wishes to combat possible effects of carry over.

The most striking effect was detected with restraint cones. The responses of cardiovascular parameters were largest and their duration longest. The restraint cones are designed to hold and restrain rodents for injections (*Hau & Van Hoosier, 2003*), and as such are suitable for use by inexperienced personnel; but it appears that this method is quite disturbing to rats. One tentative explanation could be that the rat's whiskers are squeezed along the head when they are within the restraint cones.

Rats rely heavily on their whiskers, even more so than on vision. Whiskers are in constant back and forth movement and the sweeping frequency depends on what they are doing (*Semba & Egger, 1986; Fanselow & Nicholelis, 1999*). Rats create a three dimensional picture of their surroundings by tapping objects with their whiskers and specifically, use their whiskers to determine whether or not they can fit through an opening (*Schiffman, 1970*). With this in mind, it is understandable that placing a rat snugly into a restraint cone can be disturbing to them.

Tilting the long axis of animals can and has been used to study the orthostatic reflex - tilting the head of conscious, normotensive rats by up 45° for minutes evokes hypertension as detected with telemetry (*Raffai et al., 2005*). When immobilizing rats either with the scruff or by encircling, the long axis of the rat body is vertical, i.e. a tilting angle of about 90°. This study used a rather short exposure to handling, but even that resulted in an increase in blood pressure, that itself would have narrowed the difference in blood pressure detected between scruff and methods of horizontal posture, i.e. cone and holding by tail.

To estimate the differences between repeated handling, data from the first day were compared with that from two consecutive days immediately after the designated handlings. The parameter values as such did not change after repeated handling procedures, but the response duration showed significant decreases on the next two days after scruff handling (Figure 8). Consequently, holding by the scruff can be considered as the best method of handling of those assessed in this study. One tentative explanation is that it elicits a reflex with pleasant memories from the neonatal period when pups are carried by their mother.

Dorsal immobility, or the transport immobility response, is triggered when rats are picked up by the scruff, i.e. constriction of the skin at the nape (*Mileikovsky & Nozdrachev, 1997*). This is seen in juveniles when the mother picks them up for transport (*Wilson & Kaspar, 1994*). However, the response persists into adulthood, as adult rats do freeze when they are picked up in this manner (*Webster et al., 1981*).

In conclusion, this study shows that there is refinement potential in routine handling procedures and it really makes a difference which handling technique is selected. Manual handling seems to be preferable over device-based techniques; yet it must be emphasized that mastering the handling technique to be used is of prime importance. Handling rats before the test procedure may benefit also the handler since he or she will become acquainted with what to expect during the actual experiment, but repeating handling for three days in rats seems to yield habituation only with the scruff method, with about 30% shorter duration of the response seen on the second and third days, though even this will contribute considerably to the animals' well-being.

Acknowledgements

We are grateful to research technologists Tuula Mäkinen, LAC, University of Oulu and Heikki Pekonen NLAC, Kuopio, for carrying out handlings with expertise and to research technologist Veikko Lähteenmäki, LAC, University of Oulu, for taking photographs for this article.

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