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The welfare impact of gavaging laboratory rats

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Abstract

Gavaging (oral dosing) has previously been shown to have only a short-term effect on behavioural parameters in the laboratory rat. The aim of this study was to determine if the gavaging of laboratory rats influenced their heart rate, blood pressure and body temperature, and if so, whether the duration of this impact correlated with the volume gavaged. The three stress parameters were measured using telemetric transponders placed in the abdomen of eight female Sprague-Dawley (Mol:SPRD) rats. Using a Latin Square cross-over design, the rats were gavaged with three different doses of barium sulphate (4, 10 and 40 ml kg^{-1}); in addition, there was a control of no dose, only insertion of the tube. The heart rate, blood pressure and body temperature of the rats were monitored continuously for 4 h after dosing and again for 1 h, 24 h after dosing. The gavaging of laboratory rats was shown to induce an acute reaction: after 30 min, blood pressure and heart rate were significantly higher than before gavaging, and body temperature was significantly higher 60 min after gavaging — indicators of stress levels comparable to those of other basic experimental procedures. A significant correlation between heart rate and dosage was observed until 10 min after gavaging. This indicates that the dosage gavaged is of only minor importance in causing stress, and only important for the most acute reaction. However, because of the resistance and discomfort observed when administering a 40 ml kg^{-1} dose, this dose should be administered only with caution.

Keywords: *animal welfare, blood pressure, gavaging, heart rate, laboratory rats, telemetry*

Introduction

Gavaging, ie oral dosing, is a common procedure in scientific experiments using laboratory rats (Morton *et al* 2001). Yet few studies have attempted to determine the level of stress induced by the gavaging procedure itself, or to determine the maximum acceptable volume to be administered if adverse effects on the well-being of the animals are to be avoided. The recommended volumes vary from 10 ml kg^{-1} up to 40 ml kg^{-1} (Hull 1995; Brown *et al* 2000; Alban *et al* 2001; Diehl *et al* 2001; Pekow & Baumans 2003), but often such recommendations appear to be based upon 'good practice' rather than scientific investigations.

In a study by Alban *et al* (2001), rats gavaged with increasing volumes of barium sulphate showed a volume-dependent effect on body temperature and behaviour. The larger the volume of barium sulphate, the lower the activity of the rats in an open-field test; in addition, body temperature, measured by scanning of a subcutaneously placed microchip, decreased with increasing volume of barium sulphate. Radiographs revealed that rats gavaged with volumes of 48 ml kg^{-1} or more always spontaneously released the barium sulphate into the duodenum, and showed signs of discomfort and cyanosis. To prevent any spontaneous release into the duodenum by enforced opening of the pyloric sphincter, rats could be gavaged with a maximum volume of just 4 ml kg^{-1}.

In a study by Brown *et al* (2000), rats were gavaged with different volumes of oil- and water-based vehicles; plasma corticosterone was measured as an indicator of stress. A significant increase in corticosterone was found only after gavaging with 40 ml kg^{-1} with the oil-based vehicle, suggesting that the type of vehicle may be important for the stress response. However, in this study by Brown *et al* (2000), some of the rats had inhaled the vehicle, which would be an obvious cause of the stress observed. Other studies have also reported complications caused by the procedure; for example, Germann and Ockert (1994) observed 32% mortality attributable to asphyxia caused by impacted food and bedding material in the oropharynx of gavaged rats; granulomatous inflammation caused by the gavaging procedure appeared to be the source of the impacts. Murphy *et al* (2001) observed 56% mortality in non-anaesthetised rats that were gavaged daily, whereas halothane anaesthetised rats had only 3% mortality. The complications observed in the non-anaesthetised rats were weight loss, incomplete vehicle retention, oesophageal impacting, haemorrhage and oesophageal perforation.

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Table 1 Mean heart rate of eight Mol:SPRD rats intra-gastrically gavaged with different volumes of barium sulphate in a Latin Square cross-over study. Difference between heart rate observed for all volumes at different time points compared with the heart rate observed 18.0 min prior to gavaging (–18.0 min) using a one-way ANOVA; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. SEM = standard error of the mean.

Time	0 ml kg^{-1}		4 ml kg^{-1}		10 ml kg^{-1}		40 ml kg^{-1}		All volumes	
(min)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
–18	340	9	323	11	329	9	350	13	336	7
0.5	436***	16	436***	9	417**	21	409*	21	425***	8
5.0	371	15	375*	16	385*	18	428**	19	391***	9
10.0	362	9	402**	18	416**	24	427**	19	403***	10
15.0	395*	18	425***	17	419**	16	422**	18	416***	8
30.0	414**	19	404**	20	404**	16	394	16	404***	9
60.0	349	16	336	11	345	16	346	9	343	6
120.0	335	19	342	15	323	9	331	12	333	7
210.0	331	14	342	17	338	20	338	13	337	8
1440.0	369	16	328	14	348	13	349	13	349	5

It is not clear from any of these studies which volume would be the recommended maximum dosage. The decreased activity in response to volume observed by Alban *et al* (2001) might have been because of a feeling of being heavier or more lethargic rather than, for example, a feeling of nausea, stomach pain or anxiety. Some of the other studies obviously had problems with the techniques used; for example, the study by Brown *et al* (2000) does not necessarily reveal the accurate impact of a correctly dosed volume as some of the vehicle was inhaled.

The aim of this study was to use telemetrically implanted transponders to investigate the stress caused by the gavaging procedure in laboratory rats, to assess the duration of the stress following the gavaging procedure and to investigate whether there was a correlation between the stress observed and the gavaging volume.

Materials and methods

Animals

This study was carried out in accordance with a license issued by the National Animal Experimentation Inspectorate after an evaluation in the Board of Animal Experimentation under the Danish Ministry of Justice.

Eight 250–300 g female Mol:SPRD rats were used in this study. Four weeks prior to the start of the study, a telemetric recorder (TL11M2-C50-PXT: Transoma Medical/Data Science International, Arden Hills, USA) was surgically implanted into the abdomen of each rat, under anaesthesia induced and maintained using isofluorane (Isoflo vet: Schering-Plough, Farum, Denmark). Before surgery, the rats were treated subcutaneously with 0.05 ml Streptocillin® Vet (250 mg Dihydrostreptomycin sulphate + 200.000 IE penicillin procaine ml^{-1}: Boehringer Ingelheim, Copenhagen, Denmark) and 0.1 ml carprofen (50 mg ml^{-1}, Rimadyl® Vet: Pfizer, Ballerup, Denmark) per 100 g body weight. A blood pressure catheter was inserted into the aorta via the femoral artery and electrocardiogram electrodes were placed subcutaneously at the intercostal muscles. During recovery, the rats were given 0.5 ml buphrenorphine (0.3 mg ml^{-1}, Temgesic®: Schering-Plough, Farum, Denmark) subcutaneously. The rats were then treated with the same doses of buphrenorphine twice per day and carprofen once per day for the next two days. Eight to ten days after surgery, the rats were housed with another female rat that had not undergone surgery. The rats were fed Altromin 1320 diet *ad libitum* (Altromin: Gentofte, Denmark) and were kept in Macrolon™ IV cages (Scanbur BK Ltd, Lellinge, Denmark) with aspen bedding (Tapvei® Oy: Kaavi, Finland). Lights were on between 0700h and 1900h.

Experimental design

Rats were gavaged with 0, 4, 10 and 40 ml kg^{-1} of barium sulphate (1 g ml^{-1} Micropaque®: Laboratoires Roche-Nicholas, France) on four experimental days; each experimental day was separated by at least one resting day. All rats had all treatments in a Latin Square cross-over experimental design, with all treatments represented on each experimental day. The four treatments were given in the same order on each experimental day (0, 4, 10 and 40 ml kg^{-1}), with the rats being gavaged in a different order on each day. The rats were not fasted prior to administration of the barium sulphate.

During the study, the rats were handled only when removed from their cage. The rats were moved, in their cage, from the animal facility to a dedicated telemetric laboratory and placed on the laboratory table upon the telemetric receivers; they remained here from the day before gavaging until the end of the study. After the rats were weighed, their telemetric recorders were switched on. The gavaging was always carried out between 1100h and 1200h. Before dosing, the barium sulphate was heated to approximately 38°C. Gavaging was carried out using probe-ended stainless-steel gastric tubes (80 × 1.5 mm, length × outer diameter). The lid of the cage was removed and a rat was

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lifted out and restrained by holding its back skin with the body freely hanging. The rat was dosed, put back in the cage, and the lid replaced securely. The 0 ml kg^{-1} group had the catheter inserted for 15 s but no volume injected. All of the other dose groups had the catheter inserted for approximately 30 s. It was necessary to handle the 40 ml kg^{-1} group for slightly longer, approximately 50 s. The gavaging was always carried out by the same experienced person.

Telemetric recording

Receiver-plates beneath the cages received the signals from the implanted transmitters, which were then transduced to a data exchange matrix correlating the blood pressure value to the atmospheric pressure and proceeding data to a computer with Notocord HEM software. Blood pressure, body temperature and heart rate were monitored for 4 h after dosing and again for 1 h, 24 h after dosing. Data were extracted at 10 time points: the first one being 18 min before gavaging and the last one being 24 h after the procedure.

Statistics

A duration of 60 s was chosen around the following time points: –18, 0.5, 5.0, 10.0, 15.0, 30.0, 60.0, 120.0, 210.0 and 1440.0 min. A Student's *t*-test was applied to test for differences between blood pressure, body temperature and heart rate before gavaging and at each time point after gavaging. Additional power calculations were performed using Minitab 14 (Minitab Inc, State College, USA). A split plot test was performed using SAS 8.2. (SAS Institute Inc, Cary, USA) to determine a significant effect of the volume gavaged on the stress parameters measured. A one-way analysis of variance (ANOVA) was performed to determine significant differences between each stress parameter and each volume gavaged at each time point. A linear regression including all time points was performed to correlate the measured stress parameters with dose volume using Minitab 14 (Minitab Inc, State College, USA).

Results

The split plot analysis revealed a significant difference in heart rate between the four dosage volumes ($P < 0.05$). This was related to a direct correlation between the heart rate and the volume dosed 5 and 10 min after gavaging, as shown by regression analysis ($P < 0.05$) (Figure 1). There was no such correlation after 10 min.

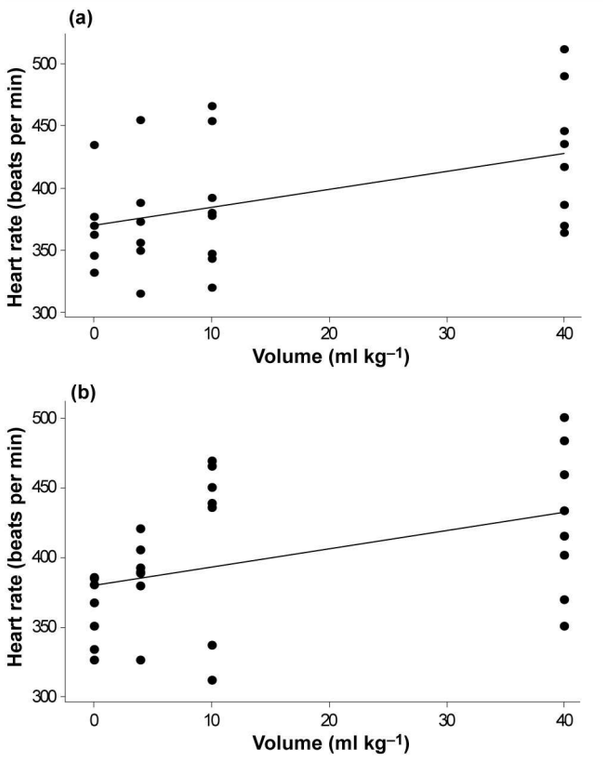
There was a significant increase in both heart rate (Table 1) and blood pressure (Table 2) for 30 min after dosing, and there was a significant increase in body temperature for 60 min after gavaging (Table 3). Power calculations for blood pressure and heart rate revealed that any differences at any time point after 30 min would be less than 6%. All of the rats increased their body weight during the study period (data not shown) and no animals died or were euthanased as a result of complications from gavaging during this study.

Discussion

Blood pressure and heart rate are normally considered to be reliable indicators of long-term stress in laboratory rats; for example, Krohn *et al* (2003) showed an increase in blood pressure and heart rate above 6% in laboratory rats in response to stressful housing conditions. In this study, blood pressure, heart rate and body temperature were all shown to be significantly elevated in rats 30–60 min after the gavaging procedure, the minimum detectable difference being 6%, ie above the impact of stressful housing shown by Krohn *et al* (2003). Therefore, it is reasonable to conclude that laboratory rats are acutely affected by the gavaging procedure and that the impact can last for up to 60 min. As 'stress' is defined as "any external stimulus that challenges homeostasis" (Moberg 1985), it could be argued that animals are stressed by the gavaging procedure; however, it should also be kept in mind that such short-term homeostasis imbalances are part of a normal life. The absolute changes in blood pressure, heart rate and body temperature are similar to changes observed in other telemetric experiments applying factors such as moving of the rats' cage, exposure to ether vapour for 1 min (Gärtner *et al* 1980) and introducing the rat into an open-field arena (Van den Buuse *et al* 2001, 2002). During this study, all of the rats increased their body weight during the study period, in contrast to the weight loss observed by Murphy *et al* (2000).

The most stressful factor is probably not the volume gavaged, but the gavaging procedure itself. As there was a positive correlation between the volume administered and the heart rate for 5–10 min after the procedure, it is reasonable to assume that the large volumes in the very acute phase affect the animal to a larger extent. This is consistent

Figure 1



Linear regression of heart rate as a function of volume dosed in rats **(a)** 5 min and **(b)** 10 min after intra-gastric gavaging. Heart rate at 5 min = 370 + 1.44 × dosed volume; $P < 0.05$, $r^2 = 21.2\%$. Heart rate at 10 min = 380 + 1.31 × dosed volume; $P < 0.05$, $r^2 = 18.0\%$.

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Table 2 Mean blood pressure (systolic pressure above diastolic pressure) of eight Mol:SPRD rats intra-gastrically gavaged with different volumes of barium sulphate in a Latin Square cross-over study. Difference between blood pressure observed for all volumes gavaged at different time points compared with the blood pressure observed 18.0 min prior to gavaging (–18.0 min) using a one-way ANOVA; *P <0.05, **P < 0.01, ***P < 0.001. SEM = standard error of the mean.

Time	0 ml kg^{-1}		4 ml kg^{-1}		10 ml kg^{-1}		40 ml kg^{-1}		All volumes	
(min)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
–18	129	3	124	3	126	3	126	4	126	2
	93	3	90	3	93	3	93	3	92	1
0.5	147**	2	146***	3	148***	3	152**	5	148***	2
	107**	2	107**	3	108**	2	111**	5	108	1
5.0	150**	3	147***	2	145**	4	153**	5	149***	2
	105**	3	103**	2	103*	4	112**	5	106***	2
10.0	145**	3	141**	2	140*	4	147*	6	144***	2
	101*	3	101**	2	101	3	109*	6	103***	2
15.0	135	3	141**	3	135	4	142*	5	138***	2
	97	3	103*	3	100	3	106*	5	102***	2
30.0	136	5	134	3	132	3	135	5	134**	2
	101	4	99	3	96	2	101	5	99**	2
60.0	130	5	122	3	126	4	124	4	126	2
	96	4	91	2	94	3	92	3	93	2
120.0	128	5	128	3	122	3	130	5	127	2
	94	4	94	3	88	3	96	4	93	2
210.0	124	3	127	4	132	5	129	6	128	2
	91	3	94	4	96	4	96	6	94	2
1440.0	131	5	126	3	125	4	130	6	128	2
	96	4	92	2	92	3	96	5	94	2

Table 3 Body temperature of eight Mol:SPRD rats intra-gastrically gavaged with different volumes of barium sulphate in a Latin Square cross-over study. Difference between body temperature observed for all volumes at different time points compared to the body temperature observed 18.0 min prior to gavaging (–18.0 min) using a one-way ANOVA; *P < 0.05, **P < 0.01, ***P < 0.001. SEM = standard error of the mean.

Time	0 ml kg^{-1}		4 ml kg^{-1}		10 ml kg^{-1}		40 ml kg^{-1}		All volumes	
(min)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
–18	37.36	0.16	37.29	0.085	37.26	0.12	37.34	0.15	37.30	0.06
0.5	37.35	0.13	37.53	0.08	37.51	0.10	37.66	0.13	37.36	0.17
5.0	37.46	0.14	37.61*	0.10	37.53	0.09	37.39	0.11	37.49*	0.05
10.0	37.74	0.14	37.87**	0.09	37.73**	0.09	37.58	0.14	37.74***	0.06
15.0	37.96**	0.12	38.03***	0.09	37.84**	0.10	37.76	0.16	37.90***	0.06
30.0	37.98**	0.08	38.03***	0.05	37.80**	0.08	37.71	0.14	37.88***	0.05
60.0	37.66	0.13	37.69**	0.08	37.64	0.17	37.39	0.18	37.60**	0.07
120.0	37.30	0.07	37.24	0.10	37.25	0.09	37.18	0.13	37.25	0.05
210.0	37.10	0.04	37.25	0.12	37.25	0.07	37.00	0.21	37.15	0.06
1440.0	37.65	0.16	37.21	0.11	37.51	0.13	37.28	0.21	37.40	0.09

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with the findings of the study by Alban *et al* (2001) that larger gavaging volumes directly enter the duodenum. Therefore, very shortly after gavaging, the stomachs of the rats do not differ physically from one another. Also in the study by Alban *et al* (2001), the correlation between changes in temperature and decrease in activity, as a response to increased gavaging volume, was shown only within the first 10 min after the procedure, whereas no differences were found later. The only discrepancy between the present study and that of Alban *et al* (2001) was that we observed an increase in body temperature whereas Alban *et al* (2001) observed a decrease. However, our temperature was monitored as a core temperature whereas Alban *et al* monitored subcutaneous temperature, which may be under the influence of acute peripheral vasoconstriction, which has no impact on core temperature. Stress may be expressed as an increase or a decrease in body temperature dependent on the dominance of either a dopamine-related (Amar & Sanyal 1981) or a prostaglandin-related response (Singer *et al* 1986), but as the increase in body temperature seems to be delayed, showing a peak after 15 min, it is just as likely to be the result of increased gastrointestinal activity as the result of stress.

Brown *et al* (2000) demonstrated an elevated plasma corticosterone level up to 4 h after gavaging rats with 40 ml kg^{-1} but not with lower volumes. These rats were gavaged with oil-based vehicles, which some of the rats had inhaled, making it very difficult to compare with this study. However, it is reasonable to assume that inhalation of oil causes long-term stress in rats; therefore, it is valid to study the importance of the viscosity of the vehicle. No conclusion can be made from this study, as only barium sulphate was used. During the administration of the 40 ml kg^{-1} dose, some discomfort and resistance in the rats was observed. Similar observations were made by Alban *et al* (2001), who observed discomfort and cyanosis when the rats were gavaged with more than 40 ml kg^{-1}. The longer handling period required for the 40 ml kg^{-1} dose is a natural consequence of the larger volume to be administered; however, whether the longer handling period or the volume itself was more important in causing the observed discomfort and increased heart rate cannot be determined in this study.

Animal welfare implications

Gavaging has an acute impact on laboratory rats that may last for 30–60 min after the procedure. The impact on blood pressure, heart rate and body temperature is comparable to that of other basic experimental procedures. There is an acute volume-dependent impact on heart rate that can be shown 5–10 min after the procedure, and because of the observed discomfort in the rats when gavaged with 40 ml kg^{-1} this volume should be used only with caution. However, for gavaging volumes smaller than 40 ml kg^{-1}, it may be better for the rats to be gavaged fewer times but with a larger volume, because it appears that it is the procedure that affects the animals more than the volume. This would also decrease the risk of the complications described by others studies. Frequent gavaging probably also leads to habituation, and therefore in studies with frequent (eg daily) dosing over longer periods, the impact is likely to be reduced. It may, therefore, be less important whether animals are dosed once, twice or three times per day.

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References

Alban L, Dahl PJ, Hansen AK, Hejgaard KC, Jensen AL, Kragh M, Thomsen P and Steensgaard P 2001 The welfare impact of increased gavaging doses in rats. *Animal Welfare 10*: 303-314

Amar A and Sanyal AK 1981 Immobilization stress in rats: effect on rectal temperature and possible role of brain monoamines in hypothermia. *Psychopharmacology (Berl) 73*: 157-160

Brown AP, Dinger N and Levine BS 2000 Stress produced by gavage administration in the rat. *Contemporary Topics in Laboratory Animal Science 39*: 17-21

Diehl K-H, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal J-M and Van de Vorstenbosch C 2001 A good practice guide to the administration of substances and removal of blood, including routes and volumes. *Journal of Applied Toxicology 21*: 15-23

Gärtner K, Büttner D, Döhler K, Friedel R, Lindena J and Trautsschold I 1980 Stress response of rats to handling and experimental procedures. *Laboratory Animals 14*: 267-274

Germann P-G and Ockert D 1994 Granulomatous inflammation of the oropharyngeal cavity as a possible cause for unexpected high mortality in a fischer 344 rat carcinogenicity study. *Laboratory Animal Science 44(4)*: 338-343

Hull RM 1995 Guideline limit volumes for dosing animals in the preclinical stage of safety evaluation. *Human & Experimental Toxicology 14*: 305-307

Krohn TC, Hansen AK and Dragsted N 2003 Telemetry as a method for measuring impacts of housing conditions on rats. *Animal Welfare 12*: 53-62

Moberg GP 1985 Biological response to stress: key to assessment of animal well-being? In: Moberg GP (ed) *Animal Stress* pp 27-49. American Physiological Society: Bethesda, Maryland, USA

Morton DB, Jennings M, Buckwell A, Ewbank R, Godfrey C, Holgate B, Inglis I, James R, Page C, Sharman I, Verschoyle R, Westall L and Wilson AB 2001 Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group. *Laboratory Animals 35*: 1-41

Murphy SJ, Smith P, Shaivitz AB, Rossberg MI and Hurn PD 2001 The effect of brief halothane anaesthesia during daily gavage on complications and body weight in rats. *Contemporary Topics in Laboratory Animal Science 40(2)*: 9-12

Pekow C and Baumans V 2003 Common nonsurgical techniques and procedures. In: Hau J & Van Hoosier GL (eds) *Handbook of Laboratory Animal Science, Volume 1, 2nd Edition* p 367. CRC Press LLC: Florida, USA

Singer R, Harker CT, Vander AJ and Kluger MJ 1986 Hyperthermia induced by open-field stress is blocked by salicylate. *Physiology & Behavior 36*: 1179-1182

Van den Buuse M, Van Acker SABE, Fluttert M and De Kloet ER 2001 Blood pressure, heart rate, and behavioural responses to psychological "novelty" stress in freely moving rats. *Psychophysiology 38*: 490-499

Van den Buuse M, Van Acker SABE, Fluttert MFJ and De Kloet ER 2002 Involvement of corticosterone in cardiovascular responses to an open-field novelty stressor in freely moving rats. *Physiology & Behavior 75*: 207-215

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