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Gentled and nonhandled Wistar rats in a mildly novel open-field situation

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Introduction

In previous studies we have found differences in open-field behaviour between gentled and nonhandled male Wistar rats in stressful (bright light + loud noise, *Hirsjärvi et al.* 1990) as well as fear-evoking (dark field, *Hirsjärvi & Väliaho* 1995, in press) test situations. Gentling seems an effective way of reducing the fear of human contact in these situations while the behaviours evoked by the test situation (e.g. escape activity) remain.

However, the stimuli used in open-field tests can strongly affect animals' reactions (*Hirsjärvi & Junnila* 1986). It is not self-evident that the effects of gentling may be generalized from one type of stimulus situation to another. We wanted to know if gentling would still have a significant effect if the level of novel stimuli was low and thus fear or stress-evoking factors minimal, e.g. if the only unfamiliar stimulus was a brightly illuminated round arena.

Materials and methods

Animals and their rearing conditions

The animals were thirty male SPF Kuo:Wistar rats from the National Laboratory Animal Centre, Kuopio, Finland. At weaning they were transferred to a conventional animal room and randomly divided into groups of five. They were housed in stainless steel cages (25 × 37.5 × 20 cm, Puijon Teräs, Kuopio, Finland) with wiremesh floors and fronts and trays with brown paper below the cages. Pelleted food for rats and mice (Hankkija, Finland) and tap water were available *ad libitum.*

The rearing conditions were controlled: temperature 21–22° C, relative humidity 55–75 %, air exchange 16 times per hour. In the testing room the light-dark cycle was inverted (7 a.m.–9 p.m. dark) to enable working with the rats during their active period. A dim white light was on continuously providing an illumination of 1–5 lux to the cages. During the light period the illumination in the room was about 200 lux. The background noise due to airconditioning was about 55 dB (B scale).

Pretest care and gentling

Three weeks before testing, at the age of ten weeks, the rats were brought to the testing room. They were randomly divided into gentled and nonhandled groups and placed in two separately airconditioned cubicles. Cagemates were not separated.

Routine care was given by P.H. (4.00–4.30 p.m. daily) to habituate the rats to their future observer: water bottles and the trays with brown paper below the cage floors were changed three times a week and food crevices were filled twice a week. Cages were changed weekly. The rats of the gentled group were individually gentled by P.H. (5 minutes' gentling period per cage) twice a day on weekdays (10.00–10.30 a.m. and 4.00–5.00 p.m.). On weekends only routine check was made. The only human contact of the nonhandled rats was the weekly transfer into a clean cage.

Gentling was carried out on the animals' own terms. On the first days the experimenter (P.H.) let the rats sniff her hand and touched them if they allowed it; none of the rats was caught by force. Towards the end of the first week all the rats let themselves be grasped round the shoulders and be picked up to the arm. They were gently stroked and carried a few steps to habituate them to being carried to the open-field.

On the second week almost all the rats came of their own will to the arm and were standing by the front wall of the cage at the beginning of the gentling periods. They showed no different response towards the other experimenter (T.V.) who participated in the gentling procedure from the beginning of the third week.

The open-field test

The open-field was a grey, circular plastic arena (∅ 83 cm, walls 40 cm) on the floor of which was painted three concentric circles, divided by lines radiating from the middle circle into 19 equal segments (*Broadhurst* 1960). This apparatus was located in the same room as the animal cages but away from them. Illumination (about 1500 lux at the floor of the field) was provided by six fluorescent lamps 80 cm above the floor of the field.

The rats were tested on the fourth week after their transfer to the testing room, at the age of 90–100 days. Each rat was tested singly in arbitrary order for five minutes on four consecutive days (Thuesday–Friday) between 4 and 8 p.m. Cagemates were tested in succession.

The rat was carried on the arm to the field and placed on the starting segment, facing the centre. After the trial the rat was placed in a new cage to avoid contact with its untested cagements. The field was cleaned from faecal pellets and urine with hot water and wiped dry before introducing the next animal. No detergents were used because of their odours' potential disturbing effects on the rats. According to *Satinder* (1969) as well as our own observations male rats are not affected by their predecessors' odour trails on open-field.

The rats were observed directly by the two observers (P.H. and T.V.) standing on opposite sides of the field to avoid the animal's preference for either side (*McCall et al.* 1969).

The behaviours were recorded on a check sheet, latencies and durations were determined using a stop watch. The parameters scored are presented in Table 1. Observing such a broad range of parameters is not a problem because the activities are successive. Good interobserver reliability has been observed by us as well as by others (e.g. *Eriksson & Wallgren* 1967).

Table 1. The parameters scored in the open-field test.

Total ambulation – number of segments entered with two legs
Middle field ambulation – number of central segments entered with two legs
Rearing – rising on hind legs, frequency, duration, sniffing movements of nose while rearing (= exploratory type of rearing)
Middle field rearing – rising on hind legs in middle parts of the field, frequency, duration
Grooming – washing or scratching, frequency, duration
Motionlessness – staying still, frequency, duration, active (sniffing, exploratory movements of head), passive (fluffiness, crouched up position, backwards turned ears, teeth chattering)
Defaecation – number of faecal boli, number of loose stools, latency to defecate
Urination – presence of urination
Starting latency – latency (in seconds) to move from the starting segment
Vocalization – crying when removed from home cage or open-field
Teeth chattering – was teeth chattering heard or not
Rigid movements – presence of rigid or very slow movements while moving on the open-field
Darts – occurrence of sudden darts
Fluffiness – fluffy fur throughout the trial

Statistical Analysis

Analysis of variance for repeated measurements (MANOVA and ANOVA, SAS Statistical Software, 6.06 VAX/VMS) was employed.

Results

Differences between the two groups were mainly observed on the first trial: the gentled rats had higher ambulation and rearing ($T(14,14) = -2.31$, $p = 0.03$ and $T(14,14) =$

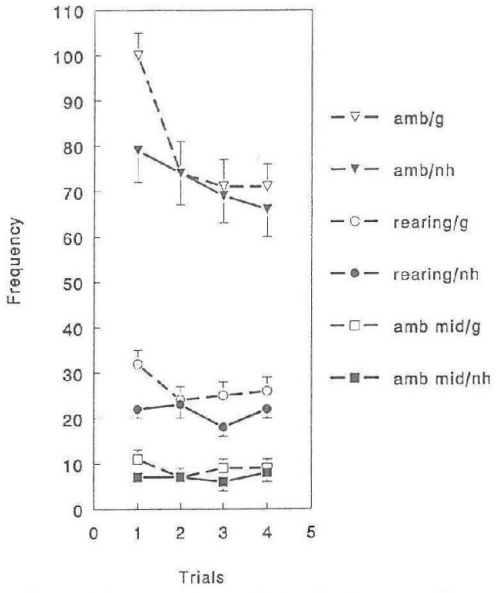

Figure 1. Frequencies and standard errors of ambulation, rearing and middle field ambulation of gentled (g) and nonhandled (nh) male Wistar rats in a mildly novel open-field situation.

– 3.11, p = 0.004, respectively) and lower passive motionlessness (T (14,14) = 2.41, p = 0.025) than the nonhandled ones. On trials two and three the gentled rats had lower defaecation frequencies (T (14,14) = 3.09, p = 0.004 and T (14,14) = 2.05, p = 0.05 respectively; Figures 1 and 2).

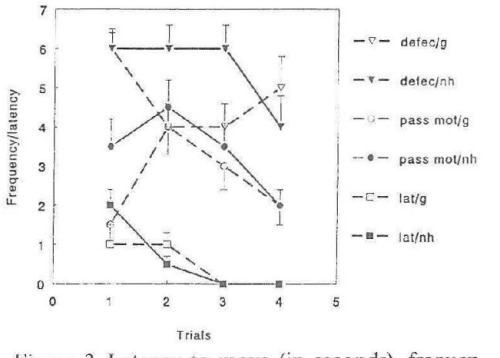

Figure 2. Latency to move (in seconds), frequencies and standard errors of defaecation and passive motionlessness of gentled (g) and nonhandled (nh) male Wistar rats in a mildly novel open-field situation.

Multivariate analysis of variance (group/trial) revealed changes in behavour as a function of trials in latency to move (F (3,84) = 23.27, p = 0.001), in ambulation (F (3,84) = 12.15, p = 0.001), in rearing (F (3,84) = 2.96, p < 0.038), in grooming (F (3,84) = 3.63, p = 0.016) and in total, active and passive motionlessness (F (3,84) = 7.72, p = 0.001, F (3,84) = 13.30, p = 0.001 and F (3,84) = 6.97, p = 0.0003). There was no interaction; the behaviours changed in similar manner in both groups.

As to changes in behaviour from trial one to trial two, ambulation decreased (T (14,14) = 2.64, p = 0.019) and passive motionlessness increased (T (14,14) = 4.00, p = 0.001) in the gentled rats. Passive motionlessness also increased in the nonhandled rats (T (14,14) = 2.44, p = 0.029), Figures 1 and 2.

Typical of both groups were moderate ambulation, rearing and middle field ambulation. Grooming and middle field rearing had very low frequencies, 1–2 of an average. Short (< 2 sec.) rearing comprised 70–80 % of all rearings.

Table 2. Number of rats vocalizing and having teeth chattering, fluffiness or darts on trials 1–4 (g = gentled N = 15, nh = nonhandled N = 15).

	vocalization		other	
	g	nh	g	nh
trial 1	2	4	5	15
trial 2	1	5	13	10
trial 3	–	2	9	14
trial 4	1	3	6	2

Table 3. Number of rats having loose stools, defaecating during the first minute and urinating on trials 1 and 2 (g = gentled N = 15, nh = nonhandled N = 15).

	loose stools		defae-cation		urination	
	g	nh	g	nh	g	nh
trial 1	7	8	12	6	13	11
trial 2	3	5	10	13	8	7

Numbers of rats vocalizing, showing teeth chattering, fluffiness and darts or defaccating, urinating and having loose stools are shown in Tables 2 and 3.

Discussion

The brightly illuminated open-field did not evoke strong reactions in the rats although the vocalization, teeth chattering and fluffiness observed suggest some fear (*Hirsjärvi et al.* 1990, *Hughes* 1969, *Öhman* 1985). The high proportion of loose stools together with immediate defaecation and urination point to vegetative stress reaction (e.g. *Walsh & Cummins*) on the first two trials.

The behavioural pattern seems much the same as that in our previous study in a similar situation (*Hirsjärvi & Junnila* 1986). It also agrees with the inverted U-theory of *Hughes & Beveridge* (1980): low degree of novelty evokes low activity.

Effects of gentling were seen on the first trial. The gentled rats were more active and showed less fear than the nonhandled ones. Higher ambulation and rearing on the first trial, their decrease on later trials and increase of passive motionlessness might also point to active/passive avoidance behaviour (*Blanchard & Blanchard* 1971, *Markel et al.* 1989, *Hirsjärvi & Väliaho* 1995, in press). However, the relatively low overall activity, the minor signs of fear, and the vegetative signs of stress in both groups do not support this view. The manner in which the behaviour changed on repeated trials is also against this theory. It is more likely that the behaviour of the gentled rats on the first trial reflected exploratory activity (e.g. *Walsh & Cummins* 1976).

The overall habituation pattern was similar in both groups. Fear and stress decreased as evidenced by the decrease in latency to move and in the number of rats urinating or having loose stools (*Ivinskis* 1970, *Walsh & Cummins* 1976, *Tachibana* 1980). Signs of increased exploration were not observed, indicating that the situation was not novel enough to evoke exploration (*Hughes & Beveridge* 1980).

The quantitative parameters that differentiated the two groups, ambulation and rearing, agree with other observations (*Ivinskis* 1968, *Hirsjärvi & Junilla* 1990, *Hirsjärvi & Väliaho* 1995, in press) indicating that these parameters could be useful in evaluating differences in emotionality. However, other quantitative parameters and also qualitative parameters such as passive motionlessness and loose stools should be observed for more reliable and accurate interpretation of behaviour.

Fear associated with a novel situation is an essential part of the open-field test. However, the additional fear evoked by human contact is not desirable. Although the importance of gentling seems greater in more novel and/or fear-evoking or stressful test situations it is not insignificant even in a situation where novelty is mild. Thus, gentling could be recommended as a routine procedure in open-field studies, especially when direct observation method is used. Habituating animals to handling might also be well-advised in other types of experiments where fear-reactions are unwanted. One minute of gentling per rat daily for two weeks – e.g. by an animal caretaker during routine care – should not be waste of time if it helps to eliminate one factor potentially affecting the reliability of the results.

Besides it's traditional applications in behavioural pharmacology and toxicology, the open-field test is increasingly used in evaluating the welfare of animals as expressed by their curiosity/fearlessness in a novel situation (e.g. *van Bergeijk et al.* 1990). Like in other fields, the value of the open-field test in evaluating welfare depends on the test design. In pharmacological studies the behavioural effects studied are usually so strong and clear that a straightforward, standard way of using the open-field test is accurate enough. In behavioural studies where even the finest nuances of behaviour may be crucial, the test design should be thoroughly considered. Minor differences may not emerge when the degree of novelty is low.

On the other hand, strong test stimuli may mask changes in behaviour. Also knowledge of behaviours typical of the species and strain studied, and use of a wide scale of parameters are essential for evaluation of welfare.

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Summary
Open-field behaviour of individually gentled and nonhandled adult male Wistar rats was studied in a mildly novel test situation. The gentled rats were more active and showed fewer signs of fear on the first trial. This difference gradually descended on later trials. There was no difference in the general habituation pattern of the two groups. Although the importance of gentling may be greater in more novel situations it is not insignificant even in situations where the degree of novelty is mild.

Sammandrag
Vi studerade open-field beteende av behandlade och obehandlade Wistar råttor i en moderat ny situation. De behandlade råttor var mer aktiva och visade mindre täcken av rädsla vid det första trial. Skillnaden minskades vid vidare försök. Det fanns ingen skillnad i habituering mellan de två grupper.

Yhteenveto
Työssä tutkittiin käsittelyyn totutettujen ja totuttamattomien Wistar urosrottien käyttäytymistä avokentällä lievästi uudessa testaustilanteessa (itse kentän lisäksi ärsykkeenä ainoastaan kirkas valo). Käsittelyyn totutetut rotat olivat ensimmäisellä testauskerralla aktiviisempia ja vähemmän pelokkaita kuin rutiinihoidolle jätetyt. Myöhemmillä testauskerroilla erot tasoittuivat. Tämän ja aiempien tutkimusten perusteella näyttää siltä, että joskin käsittelyyn totuttaminen on tärkeämpää stressiä tai pelkoa aiheuttavissa avokenttätilanteissa, se saattaa olle aiheellista myös muissa testitilanteissa, varsinkin jos testauskertoja on vain 1–2.

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