



## Research

## Comparison of 2 gentling programs for laboratory rats: Effects on the behavior toward humans



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## ABSTRACT

Gentling can reduce the fear reactions of young laboratory rats toward people long term. We were able to show this outcome in a previous study that involved a successful but elaborate gentling program. In the study at hand, we investigated whether a comparable positive effect on the behavior of rats can be achieved with a less time consuming (“reduced”) gentling program. We further determined whether the repeated testing had any influence over the rats’ behavior. Thirty-six female Wistar rats, 21 days old, were allocated to an experimental, control, and zero-control groups. The experimental group was gentled once a day for 10 minutes per cage in the fourth and fifth week of life. To assess the rats’ behavior toward humans, the animals in the experimental and the control groups were subjected to standardized behavior tests at the sixth, eighth, 10th, and 14th week of life and at the age of 6, 6.5, and 9 months. The animals in the zero-control group were only tested at 6 months of age, so that we could compare the habituation effect to the testing procedure. The test procedure included repeated catching of the animals, a neck grip, a hand test, and a modified open-field test (with human stressor). Five primary endpoints, which summarized the most important parameters for the assessment of “tameness” toward humans, were used for the evaluation of the results. The results were compared to those of the “intensive” gentling of the previous study. Up to an age of 4 months, the estimated differences between the experimental and control group of the “reduced” gentling program were significant ( $P < 0.05$ ), suggesting a higher level of “tameness” in the gentled rats. The control group habituated to the testing procedure and therefore achieved higher values over time. Comparing the “intensive” gentling program with the “reduced,” there were no significant differences in the primary endpoints up until the age of 22 weeks. After that, the “intensive” gentling produced better results regarding “tameness.” There was no significant effect of elapsed time between subsequent tests in the “intensive” gentling group, whereas the effect of elapsed time was pronounced in the “reduced” gentling group. These results indicate that the “intensive” gentling had a more persistent effect in the absence of frequent interaction with humans than the “reduced” gentling. The reduction of the time-consuming “intensive” gentling program resulted in shortening the “tameness” effect from 6 to 4 months.

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### Introduction

Simple routine manipulations during an animal experiment can elicit stress reactions in laboratory rats if the rats are not used to humans (Kvetnansky et al., 1978; Gärtner et al., 1980; Brand, 1998; Mende, 1999; Sharp et al., 2003). Lifting and gently touching rats and fixation using a neck grip can significantly increase the corticosterone concentration in the blood (Kvetnansky et al., 1978; Mende, 1999). Sprague-Dawley rats that were subjected to

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routine handling procedures such as cage cleaning, restraint, and subcutaneous injection or transportation showed a significant increase in heart rate values after the procedure (Sharp et al., 2003). Stress can lead to changes of physiological parameters and can compromise the reliability of the test results (Shyu et al., 1987; Lawlor, 2002). Therefore, it is desirable to avoid or reduce the fear of humans in the animals not only from an animal welfare point of view but also when considering the reliability of results of animal experiments.

It has been shown that rats can become habituated to humans and that social interactions with humans can be so rewarding for rats that they can even be used as a reinforcer for a learning task (Davis and Perusse, 1988). In addition, it was shown that rats are able to distinguish between different people, and that they prefer familiar humans (McCall et al., 1969; Davis et al., 1997).

Gentling is a widely acknowledged method to influence the later behavior of laboratory rats, and numerous studies have been carried out to investigate its effects (e.g., Weininger, 1954; Weininger et al., 1954; Weininger, 1956; Candland et al., 1960; Eells, 1961; Candland et al., 1962; Hirsjärvi and Junnila, 1988; Davis et al., 1997; Maurer et al., 2008; Cloutier et al., 2012). It should be noted that the term “gentling” is interpreted differently by different authors and that the gentling programs were applied in different life stages of the rats. Therefore, the results of the different studies were not in agreement. When a gentling procedure was performed under extremely standardized conditions and the rats were stroked while being restrained, it served as a negative reinforcement (Candland et al., 1962). In most early gentling studies, however, the results showed decreased fear reactions during behavioral tests later in life as well as improved vital parameters. Only a few studies focused on the effects of gentling on the behavior toward humans specifically. Hirsjärvi and Junnila (1988), for instance, gentled male rats at the age of 10 weeks. Contrary to the behavior of the gentled rats, the behavior of the nongentled animals in the open-field test (freezing, loose stools) suggested fear toward the person performing the test. The authors believe that nongentled rats perceive humans as predators. One of our studies (Maurer et al., 2008) showed the positive effect of an “intensive” gentling program on the behavior of rats toward humans. Female Wistar rats were subjected to a gentling program twice daily for 10 minutes per cage in the fourth and fifth week of life. This involved gentling, hand feeding, and talking to the animals. Up to an age of 6 months, differences between the gentled and nongentled rats regarding the behavior during behavioral tests could be detected, indicating a higher “tameness” of the gentled animals. Furthermore, the nongentled rats showed a significant increase in tameness over time, which could be attributed to the animals getting used to repeated behavioral tests or an age-related effect.

The fourth and fifth week of life seem to be crucial for the development of proper social behavior in rats. Rats that were kept in isolation during this period had irreversible deficits in their social behavior toward conspecifics later in their life (Hol et al., 1999). Owing to these results, the fourth and the fifth week of life was chosen as the gentling period in our previous study (Maurer et al., 2008) and for the study at hand as well. The objective was to find out whether a “reduced” gentling program during the fourth and the fifth week of life could achieve a similar effect resulting in a long-term reduction of fear reactions of laboratory rats toward humans. In contrast to the gentling program of our previous study, the animals were gentled for only 10 minutes instead of 20 minutes daily—without hand feeding or talking to them. In addition, it was investigated, whether the animals of the control group that were not gentled, but subjected to a repeated testing procedure, would habituate to that and therefore achieve results indicating a greater “tameness.” Therefore, as a comparison, a zero-control group that

was neither gentled nor tested, was studied in addition. According to Morton (1968), the term “gentling” is to be understood as gentle stroking, “taming,” and this is also how “gentling” was defined in the study at hand.

## Materials and methods

### Animals and husbandry

The study was conducted on 36 female Wistar rats from 6 different litters. The animals were purchased at the age of 21 days from Charles River Company (Sulzfeld, Germany). They were housed under standard laboratory conditions (Council of Europe, 2006) in groups of 3 in Makrolon® (Ehret GmbH, Emmendingen, Germany) type IV cages with raised lids. The rats were housed with a 12 hour/12 hour light/dark cycle, and the temperature was kept at an average of 22°C ± 2°C. The light intensity in the animal room was not more than 80 lux at all times. Wood shavings (softwood granulates) were provided as bedding material. The animals were given food (ssniff R/M-H 10 mm) and water ad libitum. Division into experimental and control group was done under the aspect of genetic balancing (Rapp and Deereberg, 1987), that is, 6 siblings each were evenly distributed to 2 cages of the experimental, 2 of the control, and 2 of the zero-control group. This was done in a way that no siblings ended up in the same cage. It was determined by lot to which treatment group the animals were assigned. All rats were marked on the tail using a standard human eyeliner pencil. The marking was refreshed every week at the time of the cage cleaning and at the end of each behavioral test. Throughout the routine husbandry and experimental periods all animals were invariably lifted and carried by means of a grip around their body rather than by their tail. All routine husbandry procedures for the 3 groups were carried out by the female experimenter in an identical manner. The outfit worn during routine husbandry procedures (white lab coat, green surgical cap, mask, and shoe covers) was identical to the outfit used during the behavioral tests by the experimenter and the unfamiliar person.

One animal in the experimental group died at the age of 8.5 months, 2 weeks before the end of the experiment. No previous symptoms of ill health were noted during daily routine visual health checks. The necropsy revealed that the animal had suffered from pneumonia which may have been the cause of death.

### Gentling method

The gentling program of the 12 rats in the experimental group was started 1 day after the arrival of the animals in the facility. During gentling, a systematic “rotation system” was applied so that the same cage was not always treated first. Gentling was performed once a day (1 hour after the beginning of the light phase) for 10 minutes per cage during a total period of 14 days. No gloves were worn during the gentling procedure or during routine animal care and husbandry procedures. This was done to allow the animals to better identify the experimenter. Before gentling the animals in 1 cage, the experimenter washed and disinfected her hands. After that, she briefly rubbed her hands with fresh cage bedding to make the smell of the disinfectant less intense for the rats.

The gentling procedure consisted of touching the animals on their entire body and on the tail in a soft and gentle fashion. Care was taken to ensure that every animal was gentled for about the same amount of time. Each rat was briefly lifted after 3 and 8 minutes of the 10 minutes of gentling. Other than that, the rats were allowed to move about freely, except if they balanced on the rim of their open home cage. They were only allowed to balance on the side of the cage located closest to the experimenter. If they were

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