



# Rats seem indifferent between their own scent-marked homecages and clean cages

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## Abstract

Previous large-scale, long-term studies suggest that rat welfare is little affected by cage-cleaning frequency. Here, we investigate this further using a preference test: arguably a more sensitive welfare indicator than those used previously. Nine pairs of rats were each housed in two interconnected cages of differing cleanliness. One cage was cleaned every 3–4 days, while the other remained uncleaned for 18 days. All rats were handled at each cage-cleaning (regardless of which cage they chose) so that this aspect of the cleaning routine remained consistent. Furthermore, ammonia build-up was negligible. We could thus see whether rats prefer their own scent-marks over clean bedding, without the confounds of differential handling or the potential harm induced by ammonia. Dwelling, resting, feeding, drinking, defecation, and ammonia concentrations were compared between the two cages. None were found to differ significantly between the cages over the course of the experiment. Power tests and confidence intervals support the conclusion that the rats had no meaningful preference for self-scented, familiar areas over clean areas. A weak preference cannot be ruled out, due to the small sample and the space limitations within laboratory cages. Also, the lack of ammonia means that the threshold at which rats would start to avoid soiled areas that do generate this compound remains undetermined. Nevertheless, together with previous studies showing no clear effects of cage-cleaning frequency on rat welfare, these choice tests indicate that it is unlikely that olfactory disruption during cage-cleaning is an important welfare concern for stable non-breeding groups of rats.

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## 1. Introduction

Previous research investigating how cage-cleaning frequency affects laboratory rat welfare suggests that rats fare similarly whether under twice-weekly cleaning regimes, or when their cages are cleaned only every 2 weeks. In a 5-month long study, cleaning frequencies were compared in terms of rats' acute behavioural and chromodacryorrhoea (an aversion-related Harderian gland secretion) responses to cleaning (Burn et al., 2006b), and their long-term aggressiveness, health, chromodacryorrhoea, handleability, and lung and adrenal pathology (Burn et al., 2006a). In each case, cleaning frequency showed no clear effects on rat welfare. Furthermore, no significant effects were found in a longitudinal study investigating how pre-weaning cage-cleaning frequency affected rats' later anxiety profiles as adults (Burn et al., 2008). The one exception was that, in breeding rats, more frequent cage-cleaning slightly but significantly increased the risk that pups would be cannibalised (Cisar and Jayson, 1967; Burn and Mason, 2008); this effect appeared to be due to disturbances being more likely to occur when pups were new-born and vulnerable if cleaning was more frequent, not due to a cumulative effect on dam welfare.

Any proposed distress from cage-cleaning could be caused by disruption of the olfactory environment (Jennings et al., 1998; Koolhaas, 1999; Hansen et al., 2000), or the disturbances associated with being transferred between cages, including handling (Balcombe et al., 2004), exposure to brighter light, and increased sound levels (Gamble, 1982; Voipio et al., 2006). Here, we concentrate on the first of these possibilities: olfactory disruption. Juvenile rats appear less anxious on self-soiled bedding than on clean bedding (Adams et al., 1983; Richardson and Campbell, 1988), but whether or not the same applies for adult rats is unknown. There must presumably be a threshold above which the degree of soiling becomes excessive and potentially harmful, when rats should start to prefer clean bedding. The (space) best-studied component of cage-soiling is ammonia, although the rat's tolerance of ammonia relative to humans is not yet known. Concentrations above 100 ppm have occasionally been observed in artificial rat burrows (Studier and Baca, 1968), but can increase blinking (Broderon et al., 1976), decrease activity levels (Tepper et al., 1985), and cause respiratory problems (Serrano, 1971; Broderon et al., 1976; Gamble and Clough, 1976; Schoeb et al., 1982; Bolon et al., 1991). Lower concentrations, which are more representative of current in-cage concentrations (Hoglund and Renstrom, 2001; Burn et al., 2006a; Burn and Mason, 2008), have not been tested in rats, but mice show no significant preference or avoidance of them (Green et al., 2008). Here, we selected an animal unit known to produce low concentrations of ammonia, allowing us to investigate rats' preferences for clean or scent-marked cages over time, without the confound of ammonia building up to harmful or aversive concentrations.

In this experiment, we also separated rats' preferences for clean or soiled cages from the other disturbances associated with cage-cleaning. Behavioural observations were taken during the light and dark phases, to monitor the rats' general preferences over the whole circadian period, and a baseline was recorded when the cages did not differ (Blom et al., 1993, 1995). We used socially housed rats, not only because rats should be housed socially whenever possible (e.g. Hurst et al., 1998; Patterson-Kane et al., 2002; Sharp et al., 2002), but also because the potential welfare impacts of different in-cage olfactory environments might include social effects (e.g. territorial security or aggression).

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