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Co-species housing in mice and rats: Effects on physiological and behavioral stress responsivity

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ABSTRACT

Co-species housing of mice and rats is common practice at most breeding facilities and research laboratories, neglecting the possible effects on the animals. We investigated physiological as well as behavioral stress-reactivity in mice and rats which were either derived from a co-species or species-separated housing condition at the breeding facilities. The animals were kept under the housing condition they were used to or assigned to the opposite one. Co-species housing had a significant impact on acute stress reactivity in mice and rats but only if they were used to this housing condition throughout their lives. Moreover, the stress-effects appeared to be long lasting. Assigning animals, derived from a species-separated housing condition, to co-species housing led to chronic stress in mice and affected experimental behavior of rats.
Our findings led to the conclusion that co-species housing in mice and rats should be avoided, supporting the recommendations by the U.S. National Institutes of Health (NIH) and the Dutch Ministry of Health, Welfare and Sport (VWS). In order to support the interpretation, facilitate the reproducibility and comparability and subsequently the generalizability of experimental results, breeding facilities should at least provide detailed information about their housing conditions.

Introduction

In the wild, rats are predators of mice (O'Boyle, 1974, 1975; van Hemel, 1975; Nikulina, 1991; Calvo-Torrent et al., 1999). Under experimental conditions, exposure to rats or to rat odor induces a variety of physiological and behavioral changes in mice, such as spontaneous abortions (de Cantazaro, 1988), avoidance of scented environments (Wuensch, 1992), fewer litter sizes (de Cantazaro, 1988), endogenous opioid-mediated analgesia (Kavaliers, 1988; Hendrie, 1991; Hendrie and Neill, 1991), increased immobility (Blanchard and Blanchard, 1989; Hendrie et al., 1996), decreased food intake (Blanchard and Blanchard, 1989; Hendrie and Neill, 1991; Shepherd et al., 1992; Blanchard et al., 1993), inhibition of sexual behavior (Blanchard and Blanchard, 1989), increased heart and breathing rate, hyperthyroidism and decreased body weight (Weiner, 1992) and higher latencies to approach and consume rat-scented food rewards (Merali et al., 2003). It may thus be assumed that rat odor induces predator-stress in mice.

Despite the effects of the acute exposure to rats or rat odor only a few studies have investigated the responses of mice being housed in the same room together with rats. For example, Calvo-Torrent et al. (1999) found that mice housed next to rats for 3 weeks consumed lower levels of sucrose and spent less time in the open arms of an elevated plus maze. D'Arbe et al. (2002) observed increases in mouse sympathetic neurotransmitter release due to species co-housing conditions of 3 weeks. In sum, there is ample evidence that mice experience stress in response to both the direct and the indirect presence of rats. This phenomenon has been deployed for developing mouse models of psychosocial stress under laboratory conditions (e.g. Calvo-Torrent et al., 1999).

Notably, the effects of the presence of mice on rats are almost unknown. Stress-responses in rats are usually provoked by the exposure to cats, cat odor, restraint, foot shock, tail shock etc. A few publications (e.g. Karli, 1956; O'Boyle, 1974, 1975; van Hemel, 1975; Garbanati et al., 1983) investigate stress-related "killing-behavior" of rats directed towards mice, during experimental confrontations in one cage. Anyway, it is unclear whether co-species housing of mice and rats does affect rats at all. Physical separation of mice and rats within laboratory facilities is recommended, primarily based on the above mentioned findings in mice, in order to prevent possible predator stress in mice, in e.g. the NIH "Guide for the Care and Use of Laboratory Animals" (1996, National Academic Press, Washington,

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DC) and in the Dutch "Regulations for the Housing and Care of Laboratory Animals" (Ministry of Health, Welfare and Sport (VWS), 03.01.2002/GZB/VVB/2148400). Despite these recommendations, however, it is common practice that rats and mice are reared and housed together in one room. An inquiry at several laboratory animal suppliers about housing conditions revealed that in the majority of their breeding facilities mice and rats are raised and housed together (own, unpublished results).

One can argue that the influence of rats on mice might be reduced or even prevented if mice are adapted to the presence of rats throughout their lives. However, to our knowledge no systematic research on this topic exists. In addition, the replicability of animal models *per se* has been shown to depend on external factors such as environmental (e.g. housing) conditions (Willner, 1997). It might therefore be suspected that experimental results obtained from co-species housed animals differ from those obtained in mice and rats housed separately. In order to investigate the stress responses and possible influences on experimental behavior in both mice and rats, which were either housed together or separated from each other after being either raised and housed together or separated from each other at the breeding facility, we carried out two independent experiments. In the first experiment male rats and mice were co-species raised and housed at the breeding facility. Subsequently, the animals were either housed together or separated from each other at our own facilities. In a second experiment rats and mice were raised and housed separated from each other at the breeding facility and subsequently either housed together or separated from each other at our own facilities.

We investigated physiological as well as behavioral stress-reactivity. Since chronically stressed animals experience acute aversive stimuli as more threatening (e.g. Ducottet and Belzung, 2005), we performed a single exposure to the forced swim test (FST; Porsolt et al., 1978) as an additional acute stimulus after three weeks of either co-species or separate housing at our facilities and investigated coping styles and acute physiological responses to the FST. We investigated the responsiveness of the hypothalamic–pituitary (HPA) axis in response to the FST by measuring the plasma concentrations of corticosterone (CORT) (McEwen et al., 1997; von Holst, 1998; Hunt and Hambly, 2006). Body weight changes were used as index of chronic stress (rats: e.g. Dess et al., 1988; Marti et al., 1994; mice: e.g. Zelena et al., 2005; Chotiwat and Harris, 2006). Since thymic involution has been described as a classic index of cumulative glucocorticoid exposure, the thymus gland weights and protein contents were taken as indices of long-term physiological stress (Mukherjee et al., 2004; Kier et al., 2005; D'Elia et al., 2009). We also investigated adrenal gland protein contents and possible hypertrophy, because both are known to be robust indicators of chronic stress (e.g. Gibson and Kohtz, 1985; Marti et al., 1994; Sterlemann et al., 2008). Furthermore the activity of adrenal tyrosine-hydroxylase (TH) was measured, because this rate-limiting enzyme in the biosynthesis of catecholamines (Ely and Henry, 1978; Sun et al., 2004) indicates the repeated stimulation of catecholamine release in stressful situations (Marashi et al., 2003).

Materials and methods

Ethical note

The protocols of the experiments (DEC-DGK numbers: 2007.I.11.126 and 2007.I.11.126-1) were peer-reviewed by the scientific committee of the Department of Animals in Science & Society, Utrecht University, the Netherlands, and approved by the Animal Experiments Committee of the Academic Biomedical Centre, Utrecht, The Netherlands. The Animal Experiments Committee based its decision on 'De Wet op de Dierproeven' (The Dutch 'Experiments on Animals Act', 1996) and on the 'Dierproevenbesluit' (The Dutch 'Experiments on Animals Decision', 1996). Both documents are available online at: http://www.vet.uu.nl/nca_nl/legislation or http://wetten.overheid.nl). Further, all animal experiments followed the 'Principles of laboratory animal care' and refer to the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003).

Animals and general housing conditions

All care-taking and experimental procedures were performed by well trained members of the laboratory. The experiments were performed with naive 9-week-old male rats and mice. In experiment 1 a total of 30 male Balb/c (BALB/cOlaHsd) mice and 30 male Sprague–Dawley (Hsd:Sprague–Dawley®™ SD®™) rats, obtained from Harlan, The Netherlands, were randomly assigned to three different groups. Group 1: mice, separated from rats ($n=15$); group 2. rats, separated from mice ($n=15$), separated from mice; group 3: mice and rats housed together in one room ($n=15, 15$). All animals had been co-species housed at the breeding facility. Unfortunately, it was impossible to derive co-species housed and species-separated housed animals from one breeding facility. All contacted breeding facilities housed their animals either separated or co-species housed. In experiment 2 a total of 30 male Balb/c (Balb/cJ RjI) mice and 30 male Sprague–Dawley (RjHan:SD) rats, obtained from Janvier, France, were used and housed under exactly the same conditions as individuals in experiment 1. These animals had been housed species-separated at Janvier's breeding facility.

In both experiments the animals were kept in temperature (22 ± 2 °C) and humidity (45% to 50%) controlled rooms of the animal facilities of Utrecht University. Rat/mouse chow (CRM, Expanded, Special Diets Services Witham, United Kingdom) and tap water were available *ad libitum*.

All animals were housed under a reversed day–night schedule (lights on between 19:00 and 07:00 h). During the 3-week pre-experimental period, the animals were habituated to handling once per day (about 2 min/animal) between 10:00 and 14:00 h, 5 days/week in order to minimize the possible influence of experimenter presence and handling on experimental outcomes. This pre-experimental period should be long enough to enable the animals to adapt to the light schedule since it is known that e.g. mice require 4 or 5 days to fully adjust to a 10-h shift in lightning conditions (Masubuchi and Sassone-Corsi, 2009). All members of the laboratory involved in the experimental procedures handled the animals. Rats and mice housed separated from each other were handled on alternating days in order to avoid olfactory stimulus transfer between the species.

Mice were individually kept in Eurostandard type II L cages ($365\times207\times140$ mm) and provided with a cardboard shelter and tissue (KLEENEX® Facial Tissue, Kimberly-Clark). Rats were individually kept in Eurostandard type III H cages ($425\times266\times185$ mm) and provided with a transparent amber-colored tunnel (Plexx® B.V., The Netherlands) and tissue (KLEENEX® Facial Tissue, Kimberly-Clark). All cages were provided with bedding material (Aspen chips).

Experimental procedures

Behavioral investigations

The forced swim test (FST)

Rats and mice were behaviorally tested in the FST. Rats were forced to swim for 5 min in a glass cylinder (experiment 1: 66 cm high × 20 cm in diameter; experiment 2: 70 cm high × 35 cm in diameter) in front of a black screen, containing water up to 30 cm height at a temperature of 21 ± 1 °C (Detke et al., 1995). Mice were forced to swim for 5 min in a glass cylinder (40 cm high × 20 cm in diameter) containing water up to 15 cm height at a temperature of 21 ± 1 °C (Crawley, 2000).