Rats Show Aversion to a Gradually Increasing Concentration of Carbon Dioxide

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Introduction

Carbon dioxide (CO_2) is used to euthanize or stun a number of different farm and laboratory species, including pigs, poultry and rodents. Evidence suggests that these species find CO_2 aversive, but the strength of this aversion in rats has not been tested.

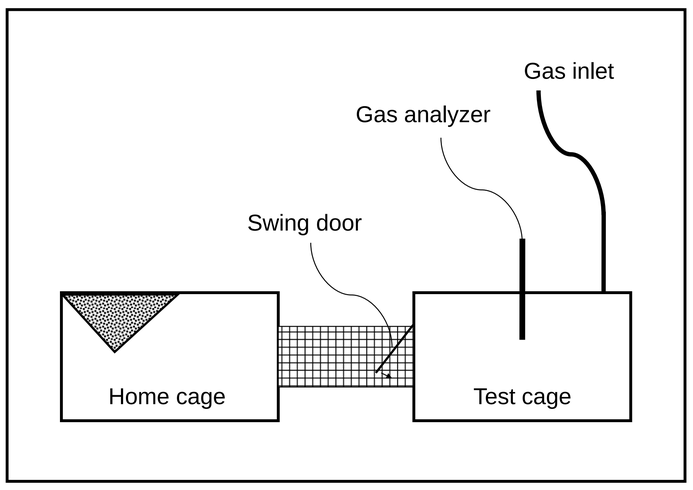
The objective of this study was to use an approach-avoidance paradigm to examine rats' aversion to an increasing concentration of CO_2.

Materials and Methods

Animals and Housing:

The subjects were 11 Sprague-Dawley rats, housed either singly (*n* =3) or in pairs (*n* = 8) in two cages connected by a wire-mesh tunnel. They had access to both cages at all times. The "home" cage contained bedding, food, water and a nestbox, and the "test" cage contained only bedding (Figure 1).

Figure 1: Experimental Set-up



Experimental Procedure:

The rats were trained daily to enter the test cage for 120 s of access to chocolate and seeds. Immediately upon entry, a swing door was closed and air flow was initiated at a rate of 20% of the cage volume per minute. Rats could re-enter the home cage at any time by pushing through the swing door. On the test day, air flow was replaced with CO_2. We recorded the elapsed time before each rat 1) left the food reward, and 2) pushed the swing door on the test day and on the three days prior (control days).

Statistical Analysis:

Analyses were performed using pair averages for pair-housed rats and raw values for single-housed rats. There were no obvious differences between pair and single-housed rats, therefore the data were pooled. Since all rats remained in the test cage for the entire 120 s session on control days, we compared the difference between the test day values and 120s using a one-sample t-test. Data are presented as mean ± SEM.

Results

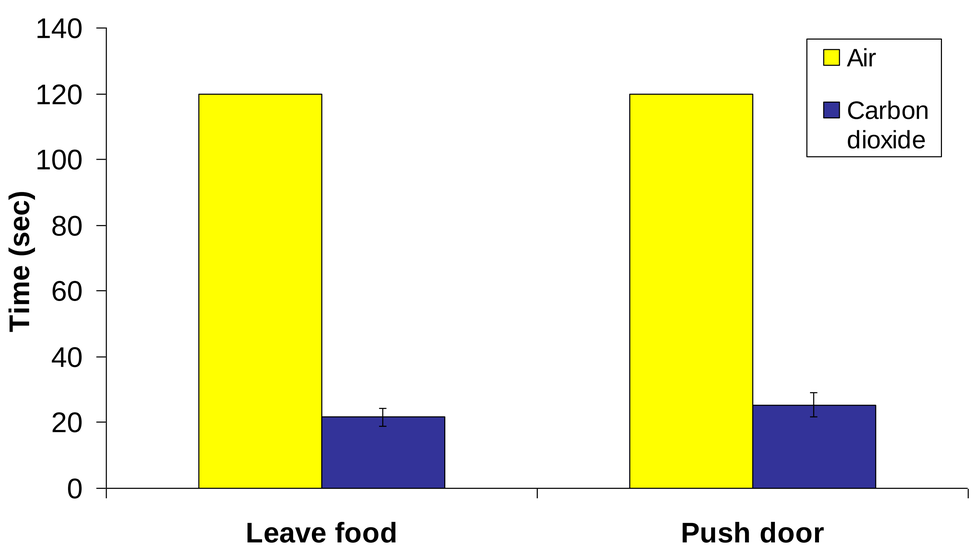


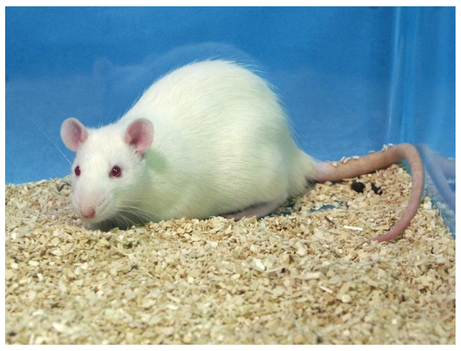
Figure 2: On the CO_2 test day, rats left the food reward and pushed on the swing door significantly earlier than on the control days (t-test: n = 7; t = 25.7 and t = 34.6 respectively; $p < 0.0001$).

Concentrations of CO_2 in the test cage at these times were 5 ± 1% and 6 ± 1%, respectively.

Conclusions

This experiment demonstrates that rats are willing to leave a food reward in order to avoid CO_2 exposure. This occurred even when the concentration was gradually increasing and at a low level.

In previous experiments we have found that CO_2 concentrations of >25% are necessary to result in unconsciousness in rats (unpublished data), and these rats only tolerated concentrations of ~6% CO_2. This indicates that both pre-fill and gradual fill CO_2 euthanasia are likely aversive to rats.



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