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# Effects of experimentally necessary changes in husbandry on olfactory memory: Chronic food restriction and social isolation

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## HIGHLIGHTS

- We examined the effect of social isolation and food restriction on olfactory memory.
- Chronic food restriction increased olfactory memory duration and specificity.
- Social isolation decreased olfactory memory duration and specificity.

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## ABSTRACT

Changes to typical procedures in animal husbandry are often necessary to accommodate the needs of behavioral experiments. Two common changes in husbandry for rodents are light chronic food restriction (to motivate animals in reward-association tasks) and social isolation (to accommodate individual feeding schedules or need to reduce interactions because of implants for example). Each of these intervention individually has been shown to modulate behavioral state and with it performance in behavioral tasks. We here systematically test how social isolation and light chronic food restriction modulate olfactory memory in rats. Our results show a strong modulation of olfactory memory after both types of husbandry interventions. These results suggest that common changes in animal husbandry promote distinct and relevant changes in animal behavior.



## 1. Introduction

The demands of a variety of behavioral tasks tend to be solved using simple changes in animal husbandry. However, the non-specific effects of these changes are generally ignored. For instance, behavioral conditioning experiments commonly use food restriction to motivate animals to learn a stimulus-reward association. Along with encouraging food consumption and associations with reward in operant conditioning tasks, food restriction can also modulate animals' performance in learning tasks independent of food associations such as the Morris–Water maze or passive-avoidance tasks (see for example [20–22,39]. In addition to classical learning and memory tasks, food restriction has also been shown to enhance olfactory discrimination [13] and memory in mice [22].

Another common husbandry change during classical learning and memory tasks is social isolation. Animals often need to be isolated in order to reduce interactions with implants or accommodate individual feeding schedules. Early life social isolation in rodents has been shown to trigger behavioral changes such as hyperactivity, aggression, and impaired spatial learning and working memory [5,24,42,44]. These changes are often accompanied by changes in development of limbic brain structures such as the medial prefrontal cortex and hippocampus [4,29,33,36,40]. To the best of our knowledge the effects of social isolation on olfactory processing have only been shown to influence processing of socially-related odors. For instance, a recent study showed that isolated mice have depressed social odor memory but not necessarily altered non-social memories and/or olfactory discrimination [18]. Brief social isolation in very young rodents was also shown to modulate the duration of social memories [3].

In the present experiment, we address the effect of these common husbandry adjustments on olfactory memory and processing using a non-invasive, non-associative olfactory learning paradigm. This task can test the formation, specificity, and duration of a simple olfactory memory [8,32,34,43]. Importantly, we use monomolecular odorants commonly used in behavioral studies rather than social or food odors to ask how processing of non-behaviorally relevant odors is modulated. First, chronic, mild food restriction, while leaving memory formation unaffected, increases both memory duration and specificity. Social isolation, likewise, had no effect on memory formation, but both memory duration and specificity were decreased in isolated rats. The observed

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changes are reminiscent of those triggered by direct manipulations of olfactory bulb function.

## 2. Methods

### 2.1. Behavioral methods for both experiments

#### 2.1.1. Experimental setups

For olfactory experiments the rats were placed in a in a 36″ × 36″ × 18″ Plexiglas open top chamber (Fig. 1). Odors were introduced using Eppendorf Tubes containing 60 μL of the desired odorants and placed into custom made holders on the base of the chamber.

#### 2.1.2. Odor recognition task

Our odor recognition task is derived from classical object recognition tasks. The object recognition task measures a simple non-associative memory without shaping or training (reviewed in [1]). Animals are presented with an object and allowed to investigate this object for a given amount of time; they are subsequently presented with the same object in the presence of a second, novel object. The relative investigation time of the novel compared to the familiar object indicates if an animal remembers the familiar object. We here use an odor recognition task [19,23,32,35] to test memory duration (how long is an investigated odor remembered) and specificity (how specific is the memory to the odor).

### 2.2. Memory duration

The purpose of this first experiment was to determine memory duration. For each testing session, rats were presented with an odor and control (mineral oil) during a 5 min familiarization or encoding trial. After a delay of 15, 30, 45 or 60-min, during the recall trials (5 min), rats were presented with the same odor (familiar odor) and an unrelated second odor (novel odor) (Fig. 1A). In this paradigm, memory for the familiar odor is considered to be present when rats investigate the familiar odor significantly less during recall than during encoding, and/or if they investigate the novel odor significantly more than the recall trial (reviewed in [1], also see [19]. Investigation times for each odorant were live scored using timers as well as offline using custom-made software. The order of the delay as well as the odor pairs used was pseudo-randomized and counter balanced between rats.

For analysis, the time rats spent investigating the familiar and novel odorants during the encoding and recall trials was used in a repeated measures ANOVA with experimental group and ITI (30, 45 and 60 min in the food restriction experiment and 15, 30, 45 and 60 min in the social isolation experiment) as between subject effects and investigation

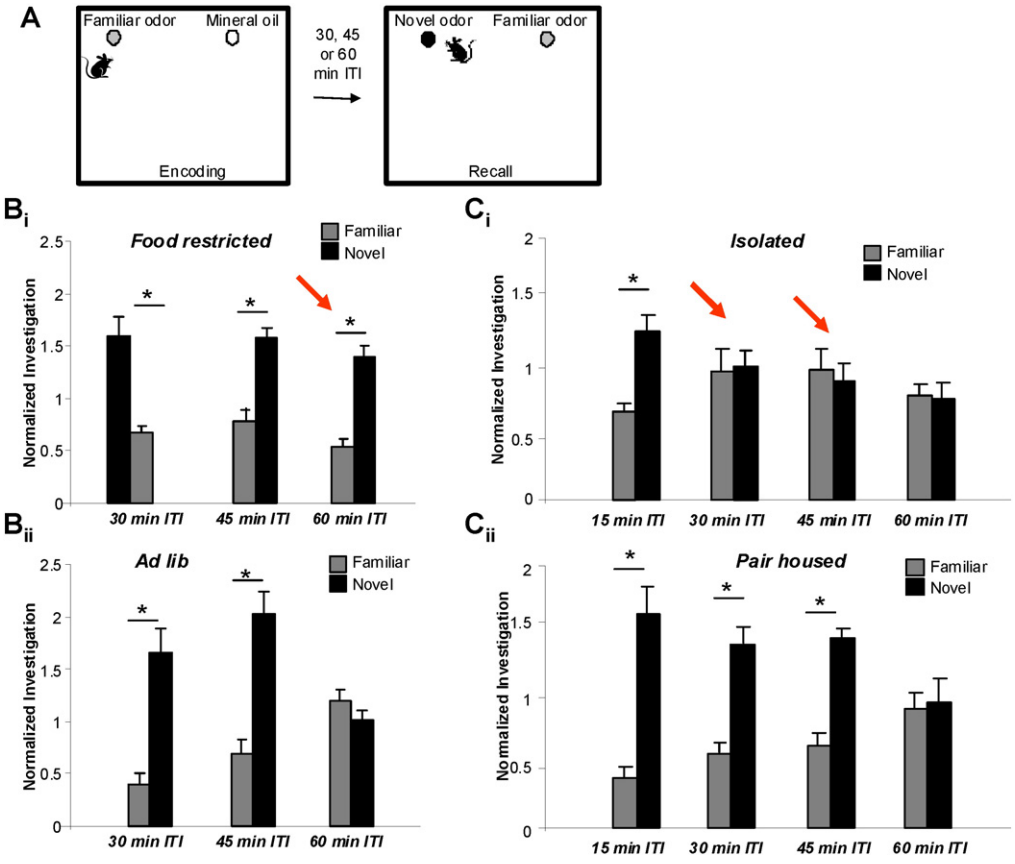


**Fig. 1.** Effect of food restriction and social isolation on odor memory duration. A. During trial 1 — encoding trial — rats were placed on an enclosed arena in the presence of two Eppendorf tubes, one containing mineral oil and the second an odor. After a delay of 15, 30, 45 or 60 min, during trial 2 — recall trial — the rat was placed onto the same platform now containing Eppendorf tubes with the familiar and a novel odor. The time rats spent investigating each odor tube was recorded and scored offline. B. Effect of food restriction on odor memory. The graphs show the normalized investigation times in response to the familiar and novel odors during recall. For each rat, investigation times were divided by the average during a session to normalize for differences in overall investigation times between individuals [12]. In this paradigm, it is assumed that rats remember the familiar odor if they investigate the novel odor significantly more during recall. Both food restricted ($B_i$) and freely fed ($B_{ii}$) rats remembered the odor after 30 and 45 min, but only food restricted rats remembered the odor after the 60 min delay. *indicate a statistically significant difference in investigation times between the familiar and novel odor during recall. C. Effect of social isolation on odor memory. Pair housed rats ($C_{ii}$) remembered the familiar odor after 15, 30 and 45 min, whereas isolated rats ($C_i$) remembered the familiar odor after the 15 min delay only. *indicate a statistically significant difference in investigation times between the familiar and novel odor during recall. Red arrows highlight differences in performance between control and experimental groups.