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Suitability of the new frontier task across mouse housing population sizes

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Abstract

The new frontier task (NFT) is a behavioural paradigm for rodents that provides insight into the motivations underlying exploration. In the NFT, subjects are provided the opportunity to climb out from their homecage and explore several platforms, called 'frontiers'. Classically, two versions of the task are employed; they differ only in the illumination level of the centrally located homecage. When dimly illuminated, exploration events are proposed to be motivated by the intrinsic rewarding nature of novel stimuli and/or satiation of curiosity. In contrast, when the homecage is brightly illuminated, exploration of the still dimly lit frontiers is proposed to be motivated by fear, since bright lights are an unconditioned fearful stimuli for many small nocturnal prey, including mice. The NFT has proven to be a sensitive test for examining exploratory drive, demonstrating both selective enhancement and selective impairment of novelty-driven volitional exploration in genetically modified mice and mice subjected to behavioural interventions. A notable aspect of the NFT is that, since the actual homecage of the experimental subjects is used, multiple animals can be scored at the same time, allowing experimenters to examine social effects on exploration and to collect data more quickly. However, laboratory mice, especially when bred in-house, are often in various cage population sizes. Therefore, our aim here was to examine potential for cage-size-induced effects on performance in the NFT, in case this might be an important confound. We also included a version of the NFT in which the homecage was illuminated with dim white light (as opposed to red or bright white light), since this version of the task is also occasionally performed. In conclusion, we find that performance in the NFT is not markedly different between mice housed in groups, in pairs, or alone, irrespective of the homecage illumination level.

Introduction

The degree of drive and arousal during feelings of human curiosity are proportional to the degree of conceptual conflict between the internal representations and external evidence [1]. As such, curiosity (or the drive to pursue information, spatial or otherwise) emerges from evidence demonstrating incomplete understanding of something that is already partially understood. Curiosity is therefore facilitated by prior understanding [2].

Since spatial exploration in rodents is also likely motivated by the inherent rewarding nature of novelty and a desire to complete an understanding of the environment, the motivation for rodents to explore novel, safe space is conceptually similar to human curiosity and may be governed by homologous molecular and cellular processes [3] [4] [5]. Therefore, rodents may exert a higher drive to explore a novel change to an already familiar environment, when compared to their drive to explore an environment that is entirely novel.

The new frontier task (NFT) capitalises on these concepts by providing spatial novelty to the already familiar homecage and by allowing mice to explore four platforms, or 'frontiers', placed along the cardinal axes. These frontiers are made accessible to the mice with small ladders that protrude partly into the homecage (see Fig. 1A). In the NFT, the homecage provides immediate partial understanding of the paradigm's spatial aspects.

To distinguish between different motivations to explore, the homecage during the NFT

can be either dimly or brightly illuminated. When the homecage is dimly illuminated, exploration is presumed to be novelty driven and may serve as a proxy for human curiosity. In contrast, when the homecage is brightly illuminated, performance in the NFT is presumed to assess fear-driven exploration, since bright lighting is a well-accepted fearful stimulus for small nocturnal prey, like the mouse. In both cases, the frontiers remain dimly lit. Therefore, experimental manipulations that selectively affect the degree of exploration in the dim or bright version of the task may be proposed to selectively affect novelty-driven or fear-driven exploration, respectively. Exploration of the frontiers that could be driven by other factors, such as hunger, thirst, the search for a mate, or other socio-sexual factors, is minimised by ensuring experimental subjects have free access to food and water prior to the task and, during the task, do not come in contact with scents of other mice.

The NFT has been used to examine exploratory drive in group-housed animals following genetic manipulation. Adult dentate gyrus-restricted overexpression [4] or global point mutation [5] of neuronal calcium sensor 1 (Ncs1) both demonstrate phenotypic differences exclusively when the homecage of the NFT is illuminated with dim light. Whereas Ncs1 overexpression leads to a dramatic increase in exploration when the homecage is dimly illuminated, a point mutation that destabilises the NCS-1 protein causes a selective decrease in exploration. This work, along with support from others [6] [7], is a prime basis for the notion that NCS-1 plays a key role in the drive to explore novel, safe space.

The NFT has also been used to demonstrate selectivity within the amotivation pathophenotype associated with mice following psychosocial stress [8]. Like mice with destabilised NCS-1, mice subjected to chronic social defeat exhibit a profound impairment in exploration of the frontiers when the homecage is dimly illuminated. This finding highlights the particular susceptibility for novelty-driven, as opposed to fear-driven, exploration to disruption following psychosocial stress.

Since the chronic social defeat paradigm necessitates single housing and control animals exhibited a similar level of exploration in the task compared with other studies, it is clear that the NFT is suitable for use with mice across the housing population sizes typically employed in behavioural research. However, the degree to which the cage population affects exploration in the NFT has not been examined directly, and it is unclear how closely balanced separate groups need to be in order to avoid population size emerging as a potential confound.

Objective

The prime objective of this study was to address how much population size affects performance in the NFT, if at all, when using males of the black 6 mouse strain.

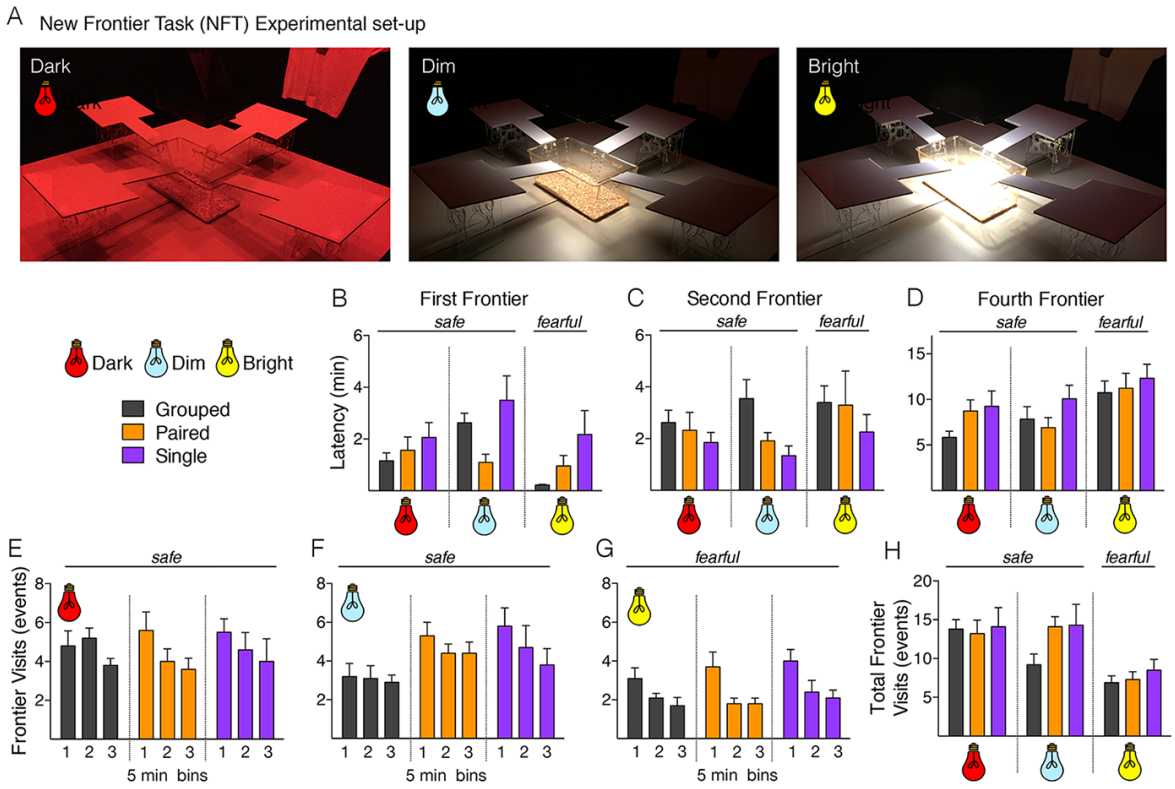

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Figure Legend

Figure 1. Performance in the new frontier task (NFT) is similar across the cage housing sizes typically required for behavioural research with mice.

(A) Experimental set-up with the 3 lighting conditions used in this experiment.

(B) Latency for the first animal of any given cage to climb out from the homecage and place all four paws on a platform.

(C) Latency for each subject to visit 2 platforms.

(D) Latency for each subject to visit all 4 platforms. A score of 15 min was assigned if not all 4 platforms were reached. Latency to the second and fourth frontier are calculated with time zero set at the time of the first frontier visit.

(E-G) Binned frontier visits in the **(E)** dark, **(F)** dim and **(G)** bright lighting condition.

(H) Total number of frontier visits during the full 15 min scoring period. For all measures, no significant effect of housing population size was observed, and no significant interaction effect was found for housing×lighting. For statistical comparisons that did reach significance, see the article's main text.

Experimental subjects

30 young adult (8 weeks of age at study onset) B6/J-Rj male mice obtained from Janvier (France). Prior to engaging in any experimental paradigms, the mice were provided with one week to acclimatise to the facility. Mice were maintained on a reverse light cycle (lights off at 7:00 am; lights on at 7:00 pm) with access to Purina mouse chow and sterile water ad libitum throughout the study. Internally ventilated cages containing tissue for nest building, pinewood bedding and a transparent plastic red house were used. After 1 week of handling, the cohort was divided equally into 3 groups- housed 5-to-a-cage, housed in pairs, and housed alone- and handling was continued for a second week before starting the NFT.

New frontier task (NFT)

Protocol and conditions were as reported previously [4] [8]. A custom made set of 4 planar T-shaped Plexiglas platforms (18 cm high, 25×20 cm) were placed around the

homecage (Fig. 1A). A small ladder (15 cm high, 5×15 cm) fixed to each platform protruded into the homecage to facilitate volitional entry to and exit from the new frontiers. Exploratory events, defined as the presence of all four paws on a given T-shaped platform, were recorded manually. The latency to the first exploration event was recorded and each trial was then proceeded for a further 15 min. In the absence of exploratory events during the first 30 min, the 15 min recording period was automatically started. 3 different illumination levels were used in order to examine novelty-driven and fear-driven exploration separately (Fig. 1A). i) Red Lighting (homecage: 15 lx) was used to provide a safe environment and the behavioural arena was exclusively illuminated with 6 equally spaced overhead red LEDs. ii) Bright Lighting (homecage: 1200 lx) was used to provide an unconditioned fearful stimulus and employed a strong white halogen spotlight hanging 12 cm above the homecage and encased in a rectangular lampshade that prevented light from illuminating the platforms. During the Bright Lighting condition, the overhead red LEDs were also on. iii) Minimal Lighting (homecage: 100 lx) was used as a control for the presence of white light and was identical to the Bright Lighting condition, except the white halogen spotlight was replaced with a 40 W white incandescent bulb. In all 3 lighting conditions, the 'new frontiers' of the NFT apparatus were illuminated almost exclusively from the overhead red lights and were ≈ 10 lx. In all trials, including those in which the homecage contained only 1 animal, each subject was given a unique marking on his tail using a felt pen to allow rapid identification by an observer when multiple animals were scored simultaneously. The dark and dim illumination levels (non-stressful versions of the task) were conducted first, and the number of subjects that received either dim or dark lighting first was counter-balanced across and within groups. The bright lighting condition was done third, as it is potentially a stressful task for the subjects.

Statistical Analysis

Binned and total exploration event data, as well as latencies to explore, were analysed using a repeated measures two-way ANOVA followed by a Bonferroni multiple correction test.

Results & Discussion

We evaluated the performance of a cohort of 30 animals in the NFT, dividing them into 3 equally sized groups house 5-to-a-cage, in pairs, or alone. We found no significant effect of the number of mice per cage ($F(2,14) = 2.910$, $P = 0.0877$) or lighting condition ($F(2,28) = 0.9309$, $P= 0.4061$) on the latency to explore the first frontier (Fig. 1AA). This was also true for the latency to explore 2 frontiers (Housing: $F(2,27) = 2.291$, $P = 0.1205$; Lighting: $F(2,54) = 1.309$, $P = 0.2785$). With respect to the latency to explore all 4 frontiers, we found no effect of housing size ($F(2,27) = 2.002$, $P = 0.1546$), but did notice a significant main effect of lighting ($F(2,54) = 6.831$, $P = 0.0023$), with bright illumination inducing a marginal increase in the latency to explore all 4 platforms. Similarly, when we examined the number of frontiers visited (exploration events), we found that all housing populations performed similarly (Fig. 1D–F), and did so irrespective of homecage illumination (Dark: $F(2,27) = 0.05980$, $P = 0.9421$; Dim: $F(2,27) = 2.335$, $P = 0.1160$; Bright: $F(2,27) = 0.5716$, $P = 0.5713$). We did however find a significant difference across time (Dark: $F(2,54)= 5.908$, $P = 0.0048$; Dim: $F(2,54) = 3.835$, $P = 0.0277$; Bright: $F(2,54) = 16.80$, $P <0.0001$), indicating that exploration events occurred more during the beginning of the 15 min testing period than at the end. This was true for all 3 lighting conditions and all 3 population sizes. When comparing the total number exploration events (unbinned), we found that lighting condition demonstrated a significant main effect ($F(2,54) = 41.68$, $P <0.0001$), with bright illumination inducing a marginal decrease in the total number of exploration events. Finally, we did not observe any significant housing×lighting interactions.

Although we did not find any statistical significance supporting differences in exploratory behaviour as a function of housing population size, we did note a trend for an increased initial latency to explore any of the 4 frontiers, particularly when the homecage was brightly illuminated (Fig. 1B). Such a finding would actually be expected given that this measure is made per cage (not per mouse). More explicitly, if the subjects in the NFT explore independently then the first exploration event should clearly

occur sooner when 5 subjects are being recorded, compared to just 2 or 1. The lack of observing a statistically significant difference here may therefore reflect social effects of group exploration, or the differences may simply lie within the expected range of variation. Modelling latencies for group-housed subjects based on the data distribution from singly-housed animals could provide insight into this question, and will be an interesting avenue for further research.

Conclusions

Performance of male black 6 mice in the NFT is similar across the cage housing sizes typically required for behavioural research with mice. Experiments comparing groups of sizes containing 10 mice or fewer are unlikely to be confounded by differences in population size.

Additional Information

Methods

Experimental subjects

30 young adult (8 weeks of age at study onset) B6/J-Rj male mice obtained from Janvier (France). Prior to engaging in any experimental paradigms, the mice were provided with one week to acclimatise to the facility. Mice were maintained on a reverse light cycle (lights off at 7:00 am; lights on at 7:00 pm) with access to Purina mouse chow and sterile water ad libitum throughout the study. Internally ventilated cages containing tissue for nest building, pinewood bedding and a transparent plastic red house were used. After 1 week of handling, the cohort was divided equally into 3 groups- housed 5-to-a-cage, housed in pairs, and housed alone- and handling was continued for a second week before starting the NFT.

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Supplementary Material

Please see https://sciencematters.io/articles/201611000019.

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ATC and BJS performed experiments. ATC, BJS and CRP designed experiments. ATC, H-II, BJS and CRP analysed data. All authors discussed the data, contributed to the manuscript and declared no conflicts of interests.

Ethics Statement

All experiments and manipulations conformed to the guidelines set by the Animal Care Commission of Switzerland and were covered under the authority of animal permit ZH170/2012. All possible measures were taken to ensure minimal pain and discomfort.

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