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# Transfer to a naturalistic setting restructures fear responses in laboratory mice

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Appropriate classification of a novel stimulus as threatening or benign depends on a repertoire of prior environmental experiences involving challenge, risk, and opportunity[1,2]. Without this library, individuals may classify harmless stimuli as dangerous — a hallmark of generalized anxiety[1,2]. In humans, insufficient exposure to uncertainty or manageable risks is associated with heightened anxiety and maladaptive fear generalization and is theorized to contribute to rising rates of anxiety in children[3–5]. Although animals in natural environments accumulate a wide range of experiences that allow them to calibrate threat assessment, most behavioral studies of anxiety rely on laboratory animals housed in static, impoverished conditions. In this laboratory context, the widely used elevated plus maze (a measure of anxiety) induces a persistent fear response in mice after a single exposure. Here we show that transferring adult mice from the lab to a large field enclosure mimicking natural mouse environments was sufficient to block the development of this fear response and restore baseline levels of anxiety behavior. A canonical rodent anxiety phenotype is thus environmentally contingent and rapidly reversible, underscoring the risks of inferring general behavioral principles from impoverished housing conditions.

The elevated plus maze (EPM) is the most widely used assay for rodent anxiety and has played an important pre-clinical role in the testing of drugs related to anxiety[6] (Figure 1A). In standard laboratory-housed mice, a single exposure to the EPM induces a persistent avoidance of open arms of the assay on subsequent trials

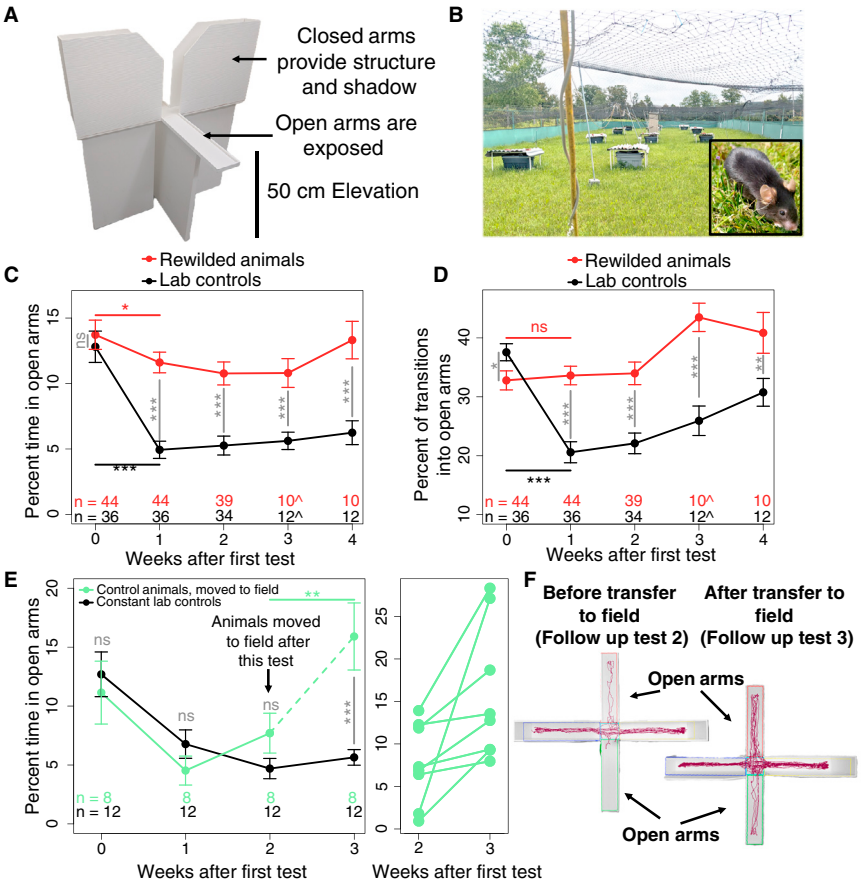


**Figure 1. Mice that live in a naturalistic field setting develop a diminished fear response to a classic anxiogenic stimulus.**

(A) The elevated plus maze is a classic assay of anxiety behavior in which mice are free to navigate between either closed or open arms. Animals that spend more time in open arms are considered less anxious. (B) A ground-level image of our naturalistic outdoor field enclosures, which are ~9,000 times the size of a standard mouse cage. Inset: a free-living C57BL/6J mouse. (C,D) Mice develop a much-diminished fear response to the elevated plus maze if they are rewilded after their initial exposure to the assay. Lab control animals develop the canonical fear response, displaying persistent avoidance of the open arms following their first exposure. ^Reduced sample sizes in follow-up tests 3 and 4 result from experimental design, not mortality. (E) Lab control animals who develop a fear response after initial exposure to the elevated plus maze and display high levels of anxiety behavior on follow-up tests 1 and 2 revert to baseline behavior after one week of living in our outdoor enclosure. Lines in the right-hand panel represent changes in time in the open arms of the assay for each of the eight mice that were re-tested. (F) A representative example of a lab control animal's movements in the elevated plus maze before and after being transferred to the enclosures, after follow-up test 2. Throughout, asterisks denote statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

('one-trial tolerance') that resists even effective anxiolytic drugs[7].

Here, we test the hypothesis that the development of this canonical rodent fear response to repeated EPM exposure is an artifact of the relatively simple and static standard laboratory housing environment. We compared adult C57BL/6J mice housed under standard lab conditions with 'rewilded' animals living in large outdoor enclosures rich in physical and social dynamism[8] (Figure 1B and experimental procedures in the Supplemental information). This design allows a direct test of whether experiencing increased and more natural environmental complexity alters the development of the fear response related to anxiety behavior by providing individuals with a larger range of reference experiences.

Exposure to a naturalistic outdoor environment blocked and reversed the anxiogenic effects of the elevated plus maze across two independent cohorts

(Figures 1 and S1 and Table S1). We replicated the canonical increase in anxiety-like behaviors in single-housed mice living in the lab after their first encounter with the EPM (Figure 1C,D, n = 36 mice). These animals showed dramatic reductions in the percentage of time that they spent in the open arms from baseline to subsequent tests (mean = 12.8% at baseline versus 4.9–6.2% in follow up tests, $p < 0.0001$ for all comparisons) and a decline in the percentage of transitions into open arms (mean = 38% at baseline, 20–32% during follow up tests, $p < 0.008$ for all comparisons).

We tested a separate group (n = 44 mice) of lab-reared mice on the EPM before releasing them into one of two large (~450 $m^2$) outdoor enclosures where they experienced a complex and dynamic environment (hereafter 'rewilded mice'[8,9]). We re-captured and tested these animals weekly, with rewilded animals continuing to live in the enclosures between tests (see Experimental Procedures). Rewilded mice displayed little to no change in their anxiety-like behaviors following repeated exposure to the elevated plus maze while they lived outside for 1–4 weeks (mean = 13.7% at baseline versus 10.8–13.3% in follow up tests 1–4, $p = 0.02$-$0.47$; Figure 1C). Similarly, rewilded animals showed no decline in the percent of entries that they made into open arms following baseline tests (mean = 33% at baseline vs 34–41% in follow up tests 1–4; Figure 1D). These results were qualitatively replicated in two independent cohorts across separate field seasons (Figure S1).

Based on this first result, it was unclear if rewilding shortly after exposure to the EPM merely blocked the anxiogenic effects of the assay or if rewilding instead changed how mice assessed the relative threats of known stimuli. To test whether rewilding also altered an already developed fear response to the EPM, we placed a subset of the lab control mice into the field enclosure following their third exposure to the EPM, after they had developed a fear response to the assay. These mice showed the classic decline in open-arm time between their first and second exposure to the EPM and maintained the same low levels in their third trial, all while they lived in standard lab housing. After spending one week in the field enclosures, these animals (n = 8) all displayed less anxiety-like behavior, and on average more than doubled the amount of time that they spent in the open arms of the EPM on their next test (from 7.7% to 15.9%, $p = 0.002$; Figure 1E,F). The anxiety behavior of these mice was not statistically different from their baseline values (15.9% versus 12.3%, $p > 0.2$). Thus, rewilding can reverse previously acquired fear responses in typically housed lab mice, indicating that anxiety-like behavior is highly plastic and environmentally contingent.

Our results demonstrate that the development of the canonical rodent fear response to an anxiogenic stimulus depends on the nature of their environmental experiences. Whether the rewilding experience is a salient treatment due to its similarity to ancestrally and evolutionarily relevant environments or simply because it has increased complexity and dynamism is unclear.

The generalizability of behavioral studies from the lab, and their potential translation to human health, relies on the assumption that animals' behavior in the laboratory environment is a reasonable proxy for behavior under other contexts[10]. The present work adds to a growing list of evidence from biological domains as diverse as development, social competition, immunology, and aging that the behavior and physiology of lab-housed mice do not necessarily generalize to more natural environments[8–10].

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## DECLARATION OF INTERESTS

The authors declare no competing interests.

## SUPPLEMENTAL INFORMATION

Supplemental information containing one figure, one table, experimental procedures, data availability, and author contributions can be found with this article online at https://doi.org/10.1016/j.cub.2025.10.050.

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