Trends in Neurosciences

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Spotlight

Studying Laboratory Mice – Into the Wild

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Studies using rewilded laboratory mice have begun to provide important clues into the complex relationship between environment, immunity, and behavior. In a recent paper, Cope and colleagues (*Hippocampus*, 2019) showed that exposing laboratory mice to outdoor living, either with or without peripheral worm infection, increased adult neurogenesis and had major effects on microglia, but only outdoor living coupled with worm infection increased anxiety.

Most laboratory rodents live in climate-controlled cages with fixed light/dark cycles and constant access to food and water, which facilitates the study of specific variables one at a time. Group housing, shelters, exercise wheels, nesting material, and toys can provide enrichment, but do not reproduce the unpredictable nature of life in the wild. The process of 'rewilding' occurs when mice are moved from their laboratory cage to an outdoor setting in which they are exposed to soil, vegetation, and insects, as well as to outdoor weather, and where they must burrow their own shelters. Similarly to wild animals, but without predation, rewilding introduces laboratory mice to several unpredictable stressors in addition to significantly enriching their experience. Cope and colleagues investigated changes of brain function and behavior in rewilded C57BL/6 laboratory mice infected with parasitic worms [1]. An earlier study found that rewilding mice before or after exposure to worms shifted their peripheral adaptive immune response and, intriguingly, modified their gut microbiome population toward worm-permissive conditions [2].

Cope and colleagues infected adult mice with the gastrointestinal nematode *Trichuris muris*, using high doses which usually trigger an inflammatory response in C57BL/6 mice, causing expulsion of nearly all parasite eggs and larvae. Ten days after infection, mice were kept in standard laboratory cages (groups of five) or rewilded in an outdoor enclosure (groups of 11–12) for ~3 weeks. Each outdoor enclosure had a surface area of 180 m^2, whereas the area of standard laboratory cages is approximately 0.05 m^2. Although rewilded mice were free to forage for food such as seeds, insects, and berries, a small shed about half the size of a standard laboratory cage also provided laboratory-grade food and water. Mice were captured weekly using long bait traps to collect stool, urine, and weight samplings. Two to three weeks after rewilding, mice were trapped a final time and tested for cognitive behavior and anxiety. After testing, animals were sacrificed to assess brain changes in adult neurogenesis and microglial reactivity. Adult neurogenesis, defined as the integration of newborn neurons into the brain circuitry, appears to be crucial for learning and memory, as well as for stress responses and cognitive flexibility [3]. Microglia are the primary brain immune cells. Their remodeling of neuronal circuits, which notably involves phagocytic elimination of newborn neurons and synapses, facilitates adaptation of brain and behavior to the environment [4]. In addition to testing for these brain changes, the authors assessed worm burden within the gastrointestinal tract.

Interestingly, and in line with earlier studies, rewilded mice had 10-fold more parasitic worms residing in their gut compared with control animals (an average of 26 worms in rewilded versus two worms in laboratory-kept mice). Studying the effects of outdoor living on neurogenesis further revealed increased numbers of juvenile neurons in rewilded mice, regardless of worm exposure. Rewilded mice had reduced numbers of microglia and diminished microglial phagocytic activity, both in naïve and parasite-infected mice. Rewilding also prevented the increase in microglial phagocytosis observed in parasite-infected mice under laboratory housing conditions. To test if the outdoor enriched environment caused any anxiety or spatial memory changes in the mice, object location memory and open-field testing were performed upon return to the laboratory. No distinct changes were seen in object location memory between mice infected or not with parasites, in either outdoor or laboratory-housing conditions. However, open-field testing revealed that infected rewilded mice spent less time in the open field compared with uninfected rewilded mice, implying that parasite infection may increase anxiety-like behavior in the wild.

The environmental enrichment of rewilding comes with several unpredictable stressors, including temperature changes, sound variability, and precipitation, as well as olfactory inputs from possible predators. With these stressors in mind, the authors tested for possible physiological and cognitive changes associated with rewilding. After a single week outside, rewilded mice gained weight at the same rate as laboratory-dwelling mice. Behavioral testing at the end of the experiment did not identify any obvious cognitive differences between naïve mice living outdoors or in the laboratory. However, further investigation is warranted to evaluate the stress response in rewilded mice, especially because recapture itself might elevate stress hormone levels, thus complicating interpretation. Less invasive experimental designs could be implemented in future studies. Because Cope and colleagues identified the rewilded mice with radio-frequency identification tags, adapted Intellicage systems could be used in future experiments to remotely monitor behavior in the wild. Anhedonia,

the lack of pleasure-seeking behavior, could be tested by measuring preference for sweetened water, and anxiety by tracking movement in open areas versus enclosed burrows of the outdoor habitat. In addition, blood levels of corticosterone, the principal stress hormone in rodents, could be measured when the mice are periodically caught to collect samplings.

In addition to environmental stressors and physical activity, rewilded mice were exposed to different food choices (such as berries, insects, and seeds) and microbes in the soil which can increase their gut microbiome diversity in as quickly as 14 days [2]. Changes in the microbiome affect the gut–brain axis and have major effects on behavior and brain physiology, including anxiety as well as microglial function [5]. Interestingly, laboratory mice tend to have immature immune systems and often die when exposed to wild mice as a result of immune challenges, but those that survive such encounters develop a more mature immune system [6]. This points to interindividual divergence in stress resilience and vulnerability that may underlie the variability seen in the behaviors of rewilded mice. Further research will be necessary to parse out the different effects of microbiota and peripheral immune changes, unpredictable stress, physical activity, and environmental enrichment on the microglial response, as well as its consequences in terms of neuronal circuitry remodeling. Manipulating any one of these variables individually, in laboratory settings, has caused variations in microglial immunological and physiological functions [4,7,8].

It is conceivable that exposure to increased physical activity in the outdoor settings, given that the mice must dig their own burrows and search for food, causes stress relief and beneficial microglial changes similar to those seen during voluntary exercise in other mouse studies [8]. In humans, experiences with nature have been shown to be strongly linked to stress relief and improved cognition [9,10].

Rewilding mice, as elegantly exemplified by Cope and colleagues in their study, may create more nuanced animal models and take environmental effects into account. Such models are instrumental in studying a wide range of pathologies driven by interplay between genetic and environmental risk factors. They may provide a window to dissect the mechanisms driving stress resilience – whether mediated by increased physical activity, sensory stimulation, or relaxation techniques – and provide better targets for effective therapies in combination with treatments aimed at modulating microglial function and stimulating neuronal plasticity.

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