Cage enrichment and mouse behaviour

Test responses by laboratory mice are unperturbed by more entertaining housing.

Mice housed in standard cages show impaired brain development, abnormal repetitive behaviours (stereotypies) and an anxious behavioural profile, all of which can be lessened by making the cage environment more stimulating[1–3]. But concerns have been raised that enriched housing might disrupt standardization and so affect the precision and reproducibility of behavioural-test results (for example, see ref. 4). Here we show that environmental enrichment increases neither individual variability in behavioural tests nor the risk of obtaining conflicting data in replicate studies. Our findings indicate that the housing conditions of laboratory mice can be markedly improved without affecting the standardization of results.

We raised female mice of two inbred strains (C57BL/6J and DBA/2) and their F_1-hybrids (B6D2F1) in three different laboratories in small, standard cages or large, enriched cages. When the mice reached adulthood, we subjected them to four of the most common behavioural tests used in drug screening and in behavioural phenotyping of transgenic and 'knockout' mice: elevated O-maze, open-field test, novel-object test and place navigation in the water maze (see supplementary information for methods). Each laboratory independently ordered three batches of 48 mice (8 mice per strain and housing condition) to run three independent replicates for each test (total number of mice was 432).

To test the effects of enriched housing on the detection and reproducibility of genetic differences in behaviour between the mice, we split the data by housing condition and calculated the proportion of variance for each replicate in behavioural measures contributed by within-group variability and by laboratory × strain interactions. Proportions of variance for representative measures of the four tests were then compared between enriched and standard housing conditions.

Figure 1 shows a summary of the results. Within-group variability contributed between 40% and 84% (average 60%) to total variance. With an average of 7.6%, the contribution of strain × laboratory interactions was considerably smaller and also less variable. However, within-group variability was unaffected by enriched housing (except for faecal bolus counts on the O-maze). This indicates that enrichment did not decrease the sensitivity of tests for detecting genetic differences and also shows that bare cages fail to remove individual differences in behaviour. Enrichment had no significant effect on the proportions of variance contributed by strain × laboratory interactions, and the direction of differences varied across measures, indicating that enrichment did not increase the risk of obtaining conflicting results between labs.

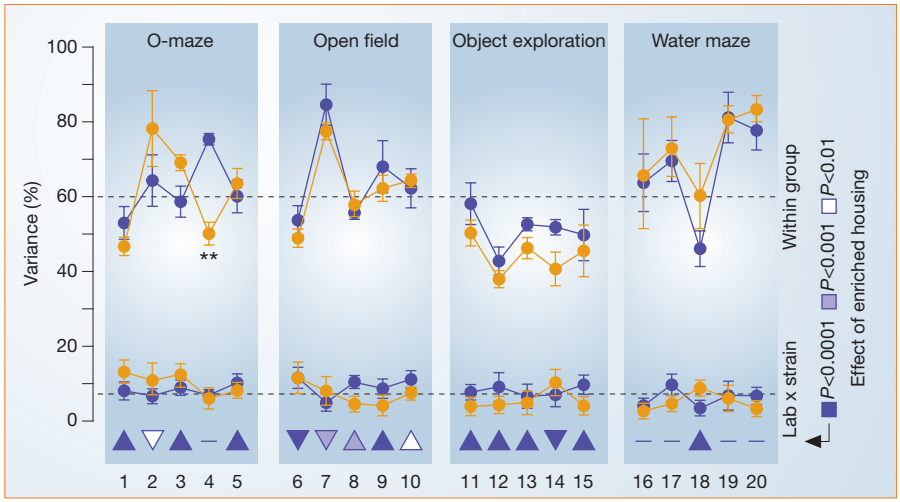

Figure 1 Mean (± s.e.m.) proportion of variance in representative measures of four behavioural tests, contributed by within-group variability and laboratory × strain interactions, in a multilaboratory study comparing female DBA/2, C57BL/6J and B6D2F1 mice housed under standard (orange) and enriched (blue) conditions. To compare the partitioning of variance under standard and enriched conditions, data were split by housing condition, and replicates were treated as independent observations. Variance proportions were arcsine-square-root transformed and compared using *t*-tests. In the object-exploration test, within-group variability of standard housed mice was decreased by a 'floor' effect (data near observable minimum) because many mice showed object avoidance. Triangles indicate direction and significance of enrichment effects on each variable. Double asterisks, significant difference in individual variability. Representative measures: 1, total head dips; 2, percentage of protected head dips; 3, percentage of open arm entries; 4, faecal-bolus count; 5, total path travelled; 6, centre avoidance, first 10 min; 7, centre avoidance, habituation; 8, path travelled, first 10 min; 9, path travelled, habituation; 10, percentage of time spent running/walking; 11, horizontal-object exploration; 12, vertical-object exploration; 13, percentage of path in object zone; 14, object-exploration distance; 15, corner distance; 16, swim-path length; 17, percentage of time spent near wall; 18, average swim speed; 19, probe: annulus crossings; 20, probe: target proximity. Animals were checked daily and remained healthy throughout the experiment. See supplementary information for further details.

Strain and housing effects on test performance were analysed using a four-way factorial analysis-of-variance model with strain, housing conditions, laboratory and replicate as between-subject factors. As in an earlier multilaboratory study[5], we found significant strain × laboratory interactions on many variables. However, these were quantitative rather than qualitative in nature, reflecting differences in effect magnitude rather than direction of the effects (see supplementary information). Likewise, enrichment effects were highly consistent across laboratories (Fig. 1, and see supplementary information).

Between-laboratory effects (contributing on average 5.2%) and replicate effects (3.1%) made comparably small contributions to total variance, indicating that standardization between labs was nearly as good as standardization within labs. This was surprising because only cage type, enrichment protocol, light phase, test system and test protocol were equated across labs. This result casts doubt on the effectiveness of excessive environmental harmonization to improve between-laboratory comparability of behavioural data[6,7] and, together with the large individual variability, underscores the need for sufficiently large sample sizes if subtle genetic effects (as often sought in 'knockout' and transgenic mice) are to be detected reliably.

Our findings argue against concerns that environmental enrichment might disrupt standardization, which may have hindered the implementation of enriched housing, despite its known advantages to animals[1–3]. Our findings should be generally applicable, for example in drug-screening or lesion studies. And they should also apply to animals' physiology or anatomy, which are, in any case, less sensitive than behaviour to environmental perturbations.

It remains to be seen whether our conclusions also apply to male mice, who may respond to enrichment with enhanced dominance behaviour and aggression[8]. But for females, environmental enrichment should improve the animals' well-being without reducing the precision and reproducibility of the data derived from them, while attenuating abnormal brain function[2] and anxiety[3] — both of which are potential confounds in animal experiments.

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Neurosurgery

Functional regeneration after laser axotomy

Understanding how nerves regenerate is an important step towards developing treatments for human neurological disease[1], but investigation has so far been limited to complex organisms (mouse and zebrafish[2]) in the absence of precision techniques for severing axons (axotomy). Here we use femtosecond laser surgery for axotomy in the roundworm *Caenorhabditis elegans* and show that these axons functionally regenerate after the operation. Application of this precise surgical technique should enable nerve regeneration to be studied *in vivo* in its most evolutionarily simple form.

Femtosecond laser pulses provide high peak intensities that reduce the energy threshold for tissue removal (ablation)[3] and enable laser surgery to be carried out with a low-energy source[4,5]. (For methods, see supplementary information.) We successfully cut single axons inside *C. elegans* by using pulse energies of 10–40 nanojoules at the specimen and tightly focused, 200-femtosecond, near-infrared laser pulses. This results in the vaporization of axon volumes of about 0.1–0.3 femtolitres, assuming an average axon diameter of 0.3 micrometres (see supplementary information). Dye-filling of axotomized neurons confirmed that the observed axon gaps are not due to photobleaching, but to physical disconnection of the axons (see supplementary information).

The minimum energy used is consistent with measured optical breakdown thresholds in transparent materials[3,6]. At these low energies, we would expect mechanical effects due to plasma expansion and shock waves to be significantly reduced[5,6] with respect to other laser-surgery techniques that require much higher energies (for example, 0.4 microjoules with 0.5-nanosecond pulses[7]). The use of pulses at a low repetition rate (1 kilohertz, 10 microwatts average power) should reduce heat accumulation and extended thermal damage to the environment. We were able to cut individual processes within a few micrometres of each other without damaging the nearby processes (see supplementary information).

The D-type motor neurons in L4 larval-stage worms were selected as targets for laser surgery. These neurons have ventral cell bodies and extend circumferential axons towards the dorsal side; they form synapses to body muscles[8]. We cut the circumferential axons (labelled with green fluorescent protein[9]) at their mid-body positions, leaving the rest of the axon intact (Fig. 1a). Both ends of the severed axons initially retracted. Among 52 operated axons (in 11 worms), 54% regrew towards their distal ends within 12–24 hours (Fig. 1b). Axons that showed partial, aberrant, or no regrowth within 24 hours did not show further improvement over longer observation times (up to 36 hours).

To evaluate functional recovery associated with nerve regeneration, we tested the behaviour of operated worms as it related to motor-neuron function. Loss of D-neuron function results in simultaneous contraction of dorsal and ventral body muscles ('shrinker' phenotype)[10], which prevents backward locomotion. Operated worms (17 in total) showed this expected 'shrinker' phenotype immediately after axotomy (15 axons per worm), whereas sham-operated animals (6 in total) moved like wild-type worms.

Remarkably, the locomotion of operated worms improved, approaching that of the wild type within 24 hours of surgery (Fig. 1c), indicating that the regenerated axons were functional (see movie in supplementary information). By contrast, the shrinker phenotype caused by laser ablation of D-neuron cell bodies did not recover after 48 hours (results not shown). The correlation of axonal regrowth with behavioural recovery in *C. elegans* indicates that these nerves must have regenerated.

Femtosecond laser axotomy is a new technique that can be performed with 100% efficiency, sub-micrometre precision and high speed. As simple organisms such as *C. elegans* have amenable genetics, application of the femtosecond laser axotomy technique we describe here should help in the rapid identification of genes and molecules that affect nerve regeneration and development.

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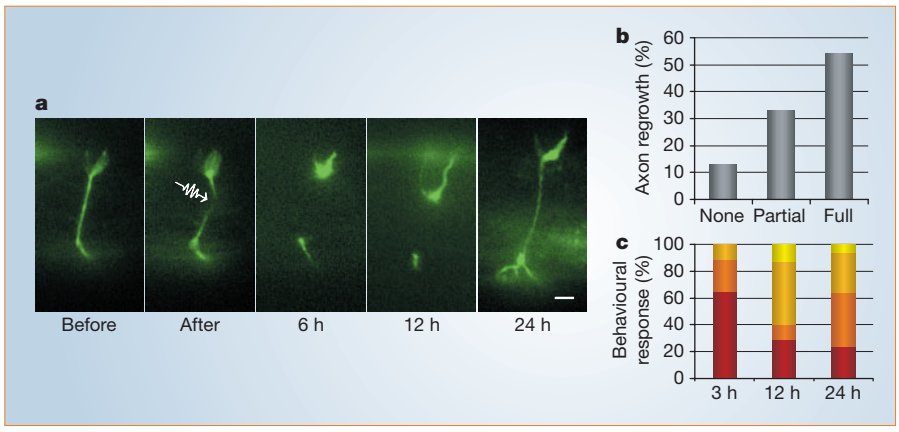

Figure 1 Femtosecond laser axotomy in *Caenorhabditis elegans* worms using 100 pulses of low energy (40 nanojoules) and short duration (200 femtoseconds) and a repetition rate of 1 kilohertz. **a,** Fluorescence images of axons labelled with green fluorescent protein before, immediately after, and in the hours following axotomy. Arrow indicates point of severance. Scale bar, 5 μm. **b,** Statistics of axon growth 24 h after axotomy, based on fluorescence images ($n = 52$ axons). **c,** Time-course analysis of backward motion of worms following axotomy. Seventeen worms were scored blindly at different time points (for criteria, see supplementary information). Improvement in backward motion was graded as four levels from 'shrinker' behaviour (dark red) up to 'wild type' behaviour (yellow) in the hours following axotomy.