aTRACKtive: Improved *in vivo* identification system for real-time, early-life individualised tracking and behavioural and welfare analysis in mice.

The scope of this Challenge has been amended to include the following:

- **The inclusion of wearables is now in scope for the Challenge, provided that, they do not cause discomfort during application or wearing, limit mobility or affect behaviour.**

All other deliverables and scope remain unchanged.

Overall aim

1. The aim of this Challenge is to establish a robust early-life mouse identification system to provide a step change in rodent behavioural and welfare analyses.

Duration

2. Up to 18 months.

Budget

3. Up to £100k.

Sponsors

4. Sponsors define the Challenges in collaboration with the NC3Rs to set out the business case and 3Rs benefits, with a view to using the product developed. Sponsors are required to provide in-kind contributions to help solve the Challenge.

5. The Sponsors for this Challenge are MRC Harwell and the National Mouse Genetics Network members Cardiff University and King's College London.

Background

6. The welfare of laboratory mice has direct impacts on the quality of research data obtained from them. A key aspect of assessing welfare is the ability to identify individual mice to understand their behaviour and social interaction when group-housed with littermates (or cage mates). It is not currently possible to identify individual mice effectively and humanely until after two weeks of age when, typically, mice are identified using ear clipping (with the tissue removed serving the dual purpose of providing material for genotyping). This means that neonatal mice welfare cannot be readily assessed and that valuable biological data that could inform the understanding of post-weaning and adult metabolism and behaviour is not collected.

7. Welfare and practical concerns associated with current options for identifying individual mice younger than two weeks of age include:

    - **Tail tipping and toe clipping** have been used in the past to individually identify neonates, however these methods are incompatible with modern welfare standards (8).

    - **Tattooing and use of a permanent marker pen** require multiple handling and the separation of pups from their nursing dam, which is stressful and could alter their behaviour. These approaches can also result in inflammatory reactions and are notoriously unreliable (9,10). For example, permanent marker pen must be frequently reapplied as it can fade over time due to grooming behaviours of the animals and tattooing can be painful, requires removal of the mice from their home cage and is technically challenging.

    - **Telemetric devices** such as radio-frequency identification (RFID) microchips are invasive and limited by both the physical size that is practical for neonatal implants and the range of current detection technologies. Larger implants with wider ranges are being used for continuous monitoring but are limited to use in older (e.g. over five weeks) mice (11).

    - **Genetic approaches** using coat colour or fluorescent identification markers are interesting alternatives but require complex breeding protocols that often result in increased animal numbers.

8. The ability to identify neonate, individual mice within a litter and monitor their behaviour remotely in the home cage could transform the way the field can explore critical early-life factors and key developmental milestones, including levels of maternal care, peer interaction and potential welfare issues (4, 5). This could also increase the potential for genetic mouse models to uncover key early indicators of disease, such as allowing the modelling of the prodromal stages in neuropsychiatric disorders (the period in which the subclinical symptoms that precede the onset of the full disorder), where there is an urgent clinical need for earlier diagnosis and intervention.

9. Identification of individual animals in a litter/cage from birth could improve the application of automated home cage monitoring using, for example, video tracking technologies. This would enable animals to be

longitudinally tracked throughout their lifetime, improving welfare and behavioural data collection. However, combining single animal identification with video tracking technologies is a key challenge. Standard physical marking methods such as ear clips have limited use as the technology is not able to reliably detect the markings. Machine learning has been used to integrate multiple camera angles to track individual pre-weaning mice in the home cage (12), but this also relies on physical marking to discriminate between animals; thus, extending it to more than two individuals in a cage remains problematic, and expanding to entire litters of very young animals is currently impossible. Older animals can be identified using RFID chips (rather than using physical marking) to provide individual readouts of activity in the home cage, which can be combined with video tracking technologies to provide measures of behaviours, but these are not suitable for use in neonates.

10. The aim of this Challenge is to develop systems to transform mouse identification from birth and that are amenable to automated tracking technology and home cage monitoring; such an advance would provide a significant impact for understanding and quantifying the life experience of an individual animal.

3Rs benefits

11. Delivery of a system that will enable individual mice to be identified from birth and tracked over their lifetime will offer the following 3Rs impacts:

12. Reduction:

    - A reduction in the number of animals required to explore the neonatal development of animals with different genotypes, since individual mice can be tracked longitudinally through the pre-weaning period, instead of separate cohorts of mice being assessed at specific time points and culled for pathology.

13. Refinement:

    - Identification and understanding of new welfare issues during development; for example, delayed feeding, drinking or motor dysfunction that allow care and interventions to be tailored accordingly.

14. The Mary Lyon Centre are running three projects, involving up to 14 national and international laboratories (many of which are in the MRC National Mouse Genetics Network (NMGN)), where this system would be transformative. Each of these projects will be characterising five to ten genetically altered mouse strains (up to 30 in total) including extensive characterisation of neonates and early developmental phases of wildtype strains, which is rarely done. A typical experiment involves up to 16 litters (around 100 pups). Over the duration of the project, this could total up to 12,000 mice.

15. This Challenge has applicability across a wide range of disciplines and sectors including in academia, CROs and pharmaceutical companies. In addition, the improved real-time tracking could be integrated to any preclinical drug study and extended into other model organisms, such as the rat. The ability to identify

mice with a system that can be used during both the light phase and dark phase would also increase the versatility of data capture options (e.g. standard out of cage testing and home cage monitoring under low light and/or infrared light).

Key deliverables

16. The aim of this Challenge is to establish a robust, non-invasive system to identify individual mice shortly after birth. The solution should last a lifetime, or at the very minimum up to adulthood, when the mice can be identified using other methods.

The approach must be:

- Non-invasive (e.g. not entering the body cavity) and non-toxic. Pups should not suffer more than mild or transient discomfort when the identification method is applied.
- Able to be used in light and dark phases (under infra-red lighting).
- Suitable for use with remote video monitoring in the home cage as well as in conventional behavioural tests such as open field and learning paradigms.
- Able to detect up to four animals individually (in one cage).
- Able to be used from postnatal day 0/1.
- Compatible with both nude pups and pups with fur of different colours, and allowing for growth of the animals (i.e. compatible with the changing physical state of the mouse as it ages).
- Require one time application without repeated handling.
- Applicable to multiple litters per day and not take more than five minutes per pup to apply.
- Compatible and integrate with standard laboratory and computing infrastructure.
- Reasonably priced to encourage widespread use.

The approach must not:

- Disturb the nursing dam once applied.
- Disturb the animal’s environment (e.g. exposure to UV light or constant lighting conditions).
- Be labour intensive.
- Involve radioactivity.
- Cause harm to, or interfere with the behaviour of, the pups or nursing dam.
- Prevent the use of bedding materials.

17. The application must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

18. The Challenge will be supported through the provision of in-kind support from the Sponsors. The in-kind support offered includes:

    - Expertise in maternal behaviour, pup social behaviour and developmental physiology.
    - Advice and ideas on device design and application.
    - Access to appropriate litters of mice as part of ongoing studies and state-of-the-art monitoring equipment including home cage monitoring systems, video footage and ultrasonic vocalisations.
    - In-house testing of a prototype device.
    - Data collection, curation and critique.
    - In-house validation with wild-type and mutant lines.
    - Access to a wide network of mouse genetic researchers for advice/analysis and critique.

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