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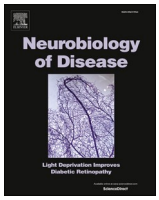

Review

The individuality paradigm: Automated longitudinal activity tracking of large cohorts of genetically identical mice in an enriched environment

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ABSTRACT

Personalized medicine intensifies interest in experimental paradigms that delineate sources of phenotypic variation. The paradigm of environmental enrichment allows for comparisons among differently housed laboratory rodents to unravel environmental effects on brain plasticity and related phenotypes. We have developed a new longitudinal variant of this paradigm, which allows to investigate the emergence of individuality, the divergence of individual behavioral trajectories under a constant genetic background and in a shared environment. We here describe this novel method, the "Individuality Paradigm," which allows to investigate mechanisms that drive individuality. Various aspects of individual activity are tracked over time to identify the contribution of the non-shared environment, that is the extent to which the experience of an environment differs between individual members of a population. We describe the design of this paradigm in detail, lay out its scientific potential beyond the published studies and discuss how it differs from other approaches to study individuality. The custom-built cage system, commercially marketed as "ColonyRack", allows mice to roam freely between 70 cages through connector tubes equipped with ring antennas that detect each animal's ID from an RFID transponder implanted in the animal's neck. The system has a total floor area of 2.74 m^2 and its spatial resolution corresponds to the size of the individual cages. Spatiotemporally resolved antenna contacts yield longitudinal measures of individual behavior, including the powerful measure of roaming entropy (RE). The Individuality Paradigm provides a rodent model of the making of individuality and the impact of the 'non-shared' environment on life-course development.

1. Introduction

In this article we describe a novel method to study how behavioral activity shapes individuality in mice. We describe our approach, termed "Individuality paradigm," against the backdrop of other emerging ideas to track individualization, mostly in laboratory animals. We emphasize what is new and different in our concept and are here not yet attempting a full, balanced review of the neurobiology of individuality and its methodology. We believe that our paradigm offers a distinct perspective on individuality that can hopefully make a meaningful contribution to the ongoing re-evaluation of variability and individuality in biological and medical research. (See Fig. 1.)

2. Enriched environments as key paradigm of unraveling gene x environment interactions

The experimental paradigm of "environmental enrichment" was developed in the early 1950s as a response to the concept of behaviorism

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prevailing at the time. Behaviorism treated the brain as a black box; and the idea of plasticity, that is structural malleability as the basis of cognitive and mental processes including learning and memory, was fundamentally foreign to this influential concept. Donald Hebb, who conceptualized synaptic plasticity, is also the father of the "enriched environment", publishing a first, rather anecdotal, report in 1947 (Hebb, 1947). The idea was intriguingly simple: give laboratory rodents exposure to more stimuli than usual and observe what happens. Where Hebb and his student Hyman had studied behavior and found that enriched rats performed better in learning tasks (Hymovitch, 1952), the "fabulous four" from Berkeley, Marian Diamond, David Krech, Edward Bennett, and Mark Rosenzweig, picked up the idea (Diamond et al., 1964) and over the following decades expanded the scope of phenotypes, including, most notably, measures of brain structure and biochemical effects (Mohammed et al., 2002). Pioneering work by Bill Greenough and colleagues extended the idea to the fine-structural analysis of neurites (Volkmar and Greenough, 1972). The tendency of all experiments was that environmental enrichment had positive effects by enhancing structure, improving functions, supporting recovery and counteracting disease. The appeal of the paradigm was gigantic. Several large reviews have summarized the core findings over the decades and have speculated about mechanisms and medical relevance (Kempermann, 2019; Mohammed et al., 2002; Nithianantharajah and Hannan, 2006; van Praag et al., 2000). We refer to these for a more detailed description of the history and key questions of this field, including the impact it had on society at large.

Importantly, environmental enrichment in its classical form has always been a cross-sectional paradigm, based on a between-group comparison. The title of an important book on the field, "Enriching heredity" by Marian Diamond, captures the prevailing interpretation (Diamond, 1988). If phenotype equals genotype plus (or times) environment, the paradigm allows assessing the impact of the environment, as long as the genetic component is controlled. Ironically, however, most early studies have been done with outbred strains of rats, so that no effective control of the genetic influence was in place. Irrespective of this, when inbred, genetically homogenous populations were used the strong effects could

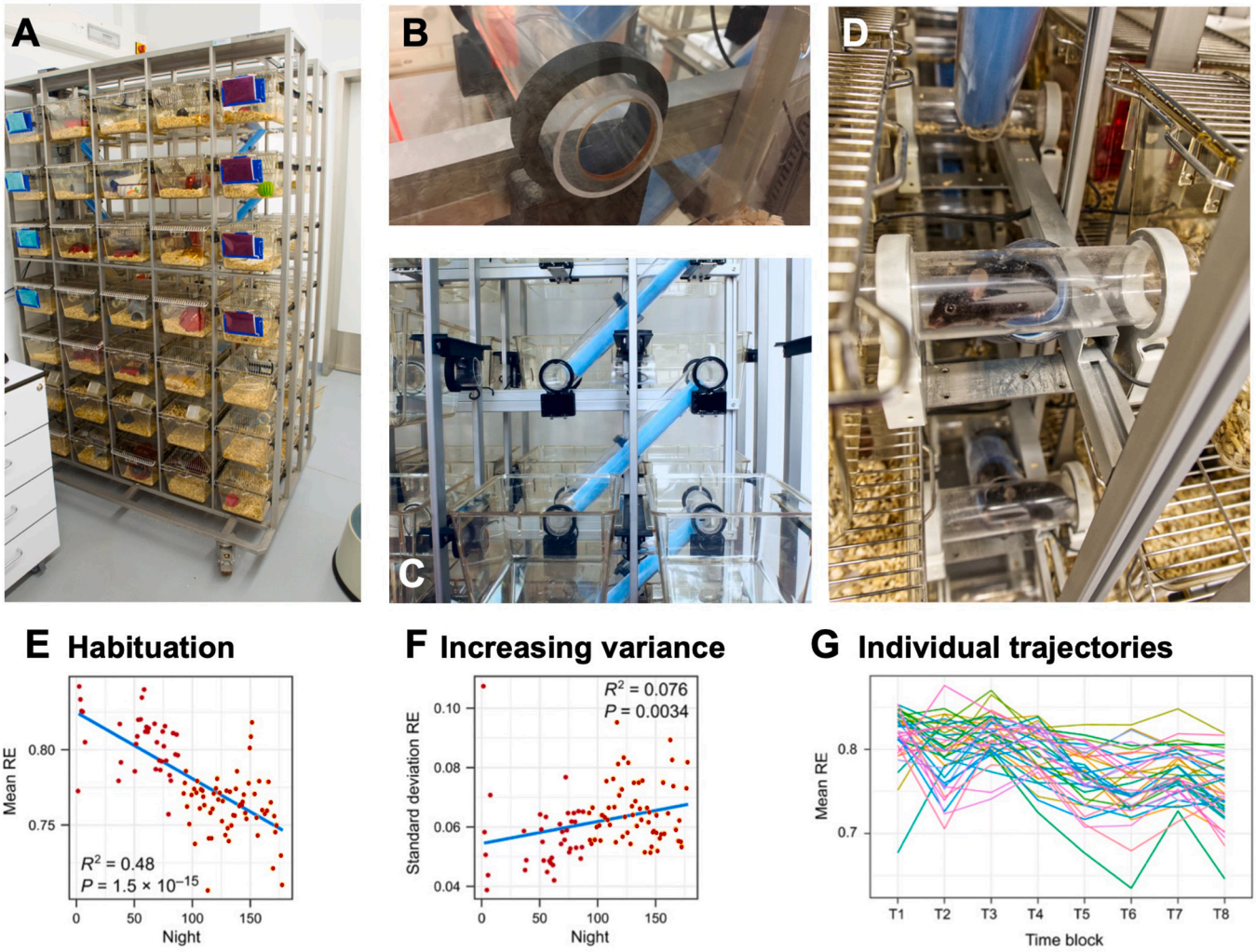

Fig. 1. The Individuality paradigm ("Colony Rack"). **A**, 3D-representation of the cage design, showing the 70 standard type II cages on a double-sided rack. The cages are connected through plastic tubes. **B**, the connector tubes are equipped with ring antennas (black rings) which collect the RFID signal from the individual transponders that the mice carry under the skin in their neck. Mice enter the tubing system through circular whole cut into the walls of the standard cages. C, tubes also connect the floor levels. The cages can be removed for cleaning. The tubes contain a removable plastic mesh that increases traction, when the mice ran the steep slopes. D, view into the tubing system with mice in action. Not that the version of the cage shown here had white ring antennas. **E – G**, exemplary roaming entropy (RE) data from a six-months study (Zocher et al., 2020). **E**, mean RE decreased over time, reflecting reduced overall activity (with considerable day-to-day variability) and is interpreted as habituation. **F**, variance in RE, however, increased over the same period of time, indicating an increasing variability in individual behavior. **G**, the individual RE trajectories displayed over 8 time brackets of the experimental period. The binning results in smoothened curves, which aids visibility of the key patterns.

still be replicated.

3. Variance can be seen as problem, nuisance, noise, or phenotype of interest

One of the notable side effects of this research was its influence on movements to improve the living conditions of laboratory animals. The positive effects of environmental enrichment, which obviously seemed to promote animal welfare, triggered questions about the extent to which elements of environmental enrichment might find entry into general husbandry of laboratory animals in order to offset some of the problems arising from their confinement to small cages and sensory deprivation (Richter et al., 2009; Van de Weerd et al., 2002; Wolfer et al., 2004). As a result, the extent to which the environmental "intervention" might work against a desired standardization began to be discussed. The general aim of conventional animal research in biomedical science is to achieve an optimal level of standardization in order to allow a clear assignment of causality and – at least in theory – increase the comparability between labs. Several studies came to the conclusion that while there might be occasional interaction effects with the phenotype of interest, by and large enrichment did more good than harm (André et al., 2018; Richter et al., 2010; Wolfinger, 2013). It was even discovered that heterogenization might actually be the cure rather than the curse and ultimately increase reproducibility (Richter et al., 2011).

The implicated belief, leading up to such studies, had been that environmental enrichment might reduce the specificity or sensitivity of the group comparison of interest, presumably by introducing a factor that increases variance. In most instances, this idea was, however, not developed beyond the speculative stage and few studies have explicitly addressed variability in the enriched environment literature (Whimbey and Denenberg, 1966). The opportunities that arise from appreciating the induction of variability as a phenotype of interest were not pursued.

Generally, however, with the rise of 'personalized medicine' there is a growing interest in phenotypic variability. With this came the insight that phenotypic variability can be leveraged to gain insight into gene x environment interactions also in humans (Marderstein et al., 2021). While this reasoning is generally also applicable to genetic reference populations in rodents, we are here dealing with the situation in which the genotype is experimentally controlled so that the environmental contribution on phenotypic variance becomes exposed.

4. The scientific context of developing the Individuality paradigm

Our own interest in environmental enrichment originated from the question, whether ethologically positive, physiological behavioral stimuli might regulate adult hippocampal neurogenesis. The background has been the idea of a 'neurogenic reserve', an activity-regulated pool of neurons enabling structural plasticity in the aging brain that might counteract otherwise occurring losses (Kempermann, 2008). Exposure to an enriched environment promotes the production, integration and function of new neurons in the hippocampal dentate gyrus of mice and rats and improves hippocampal learning and memory; including but not limited to the known functional contributions of the new neurons in behavioral pattern separation and the flexible integration of new information into pre-existing contexts. We suggest that a feedback loop exists, whose plasticity is mediated by the targeted production of new neurons that strengthen the mossy fiber connection between the dentate gyrus and area CA3 and the local network within the dentate gyrus. Existence of the feedback loop has raised the question, whether individual behaviors, through such a feedback mechanism, might result in the individualization of the hippocampal network. This would imply, however, that, given a controlled genetic background, individual behaviors in a controlled environment would increase phenotypic variance (Kempermann, 2019). That is, indeed, what we found; in our studies, the variance of adult neurogenesis often appeared to be greater in the enriched group than in the control group. We have later confirmed this impression with specifically designed studies (Körholz et al., 2018; Zocher et al., 2020). In the enriched group we find animals that hardly differ from controls, whereas others reach several-fold greater values (Freund et al., 2013; Zocher et al., 2020). The resulting Individuality paradigm, described here, allows to relate this observation of increasing variance to behavior. While we had made previous attempts to correlate levels of neurogenesis with learning (Kempermann and Gage, 2002), only longitudinal assessment of behavior would give insight into behavioral patterns that might be causal for the individualizing level of neurogenesis rather than be a covarying end-point or, by itself, a consequence of differences in neurogenesis.

By feral standards, environmental enrichment is not particularly rich and in addition the amount of change in the environment is modest. It has therefore always been a puzzle, how and why such relatively small variations induce such robust and large changes across domains and scales. The partial explanation that environmental enrichment is a highly reductionistic paradigm to reveal relative, not absolute change is one part of the explanation (Kempermann, 2019). The other, presumably here highly relevant idea is that it is actually the animal's activity in a larger social group that creates the enrichment. Across a multitude of implementations, results from the environmental enrichment literature have been very robust, which speaks to the fact that it might be a common denominator, increased group size in conjunction with more space, that induces the changes.

Home-cage monitoring is an important trend in animal studies, because it allows the undisturbed observation of the animals over longer periods of time, often regarding large numbers of physiological and behavioral parameters. Conventional tests require the animals to be transferred to a different environment, introducing confounding, often stressful influences (for a recent overview, see (Klein et al., 2022)). The "Intellicage" is a particular type of such home cage system, as it allows to behaviorally test animals on various conditioning tasks at their own pace (Galsworthy et al., 2005; Kiryk et al., 2020).

While the paradigm presented here is a home cage system, as the animals are monitored within their environment for extended periods of time, the key difference is that we describe a fully monitored enriched environment and emphasize the longitudinal assessment of emerging interindividual differences.

5. The individuality paradigm

The Individuality paradigm renders the classical enriched environment approach longitudinal, multivariate and individual (Kempermann, 2019). Although a control group is routinely maintained in parallel, the actual comparison of interest is a within-group comparison. While individual differences are typically regarded an annoyance in experimental research, our approach could be perceived as primarily a 'correlational' one (if one follows, for example (Cronbach, 1957) for a discussion of the divide). The focus of analysis is shifted to exactly assessing the magnitude of individual differences in phenotypes of interest and their associations with longitudinal (especially behavioral) trajectories. A central question of interest is, of course, on which basis one might draw causal conclusions from this experimental configuration: What is the relationship between longitudinal trajectories and endpoint measures? The Individuality paradigm shares this interest with the large number of cohort and population-based studies in humans, so that the murine paradigm, presented here, can become an animal model to analyze complex longitudinal mechanisms in a reductionist setting.

Our first study with this type of enriched environment was done in collaboration with Norbert Sachser and Lars Lewejohann from the University of Münster, who had established an RFID-based home-cage tracking system in a large enclosure (Kritzler et al., 2006; Lewejohann et al., 2009a, 2009b). RFID-based systems allow the tracking of big

cohorts of mice and thus can offer many advantages over video-based systems for home-cage monitoring. For recent examples of the opportunities of RFID tracking in mouse studies see for example (Catarinucci et al., 2014; Habedank et al., 2021; Peleh et al., 2019).

In our initial study, forty mice were tracked for 3 months living in this large enriched environment and their behavioral activity (see details below) was correlated with endpoint assessments of adult hippocampal neurogenesis. Individual levels of these longitudinal behavioral activity measures explained 22% of the variance in adult hippocampal neurogenesis (Freund et al., 2013). A first, observer-based longitudinal behavioral assessment also suggested that the mice were on increasingly stable behavioral trajectories (Freund et al., 2015).

6. The hardware of the Individuality cage system

Based on the promising results of our first enclosure but recognizing the limitations in its design (characterized by a low number of unevenly distributed antennae), we designed a new cage system with the aim of increasing the spatial resolution. We have often been asked, why we did not turn to a floor antenna system, which might provide best spatial resolution even within individual cages. But after extensive attempts with such floor antenna systems, we learned that these were prone to measurement gaps, signs of interference, and the apparent "teleportation" of mice (where animals appeared to move at unphysiological speeds; likely due to 'ghosting' artefacts in which antenna memory was not cleared correctly). In smaller settings, however, such arrangements have been successfully used (Redfern et al., 2017).

We converged on a set-up, in which 70 connected standard cages are mounted on a standard double-sided rack. The connecting tubes are equipped with RFID ring antennas that read the transponder IDs of the individual mice. The constraint space within the tunnels lowers the risk of interfering signals from two mice at the same time. The spatial resolution thus equals the size of a standard type-II cage; the overall floor area is 2.74 m^2 (without consideration of the tube area). Routinely 40 to 80 mice can live in the enclosure, where the capacity of the cage would normally be higher, but this is a good number to ensure the proper functioning of RFID recording. Food and water is usually available only on the middle level but not in the other cages. Small shelter houses like clear colored polycarbonate igloos, crawl balls, safe harbor retreats are found. To give the mice access to shelter and let them hide from dominant mice, colored polycarbonate balls of different sizes, tubes and tunnels are placed in the cages. Additionally, wood bricks, cardboard houses, chew bones, plates, ceramic bowls, swings and nesting material are provided to stimulate their explorative behavior and provide a changing environment. Toys and tubes are exchanged regularly.

The heart of the Individuality cage is the longitudinal automated activity tracking with RFID transponders that are implanted by injection under the skin in the neck of the animal. This procedure is well tolerated and tagging laboratory animals with RFID transponders is routinely done in many animal facilities. The close proximity in this design of the animal to the antenna also allows the power of the antenna to be dramatically lowered, further reducing inter-antenna interference. In the end, the multi-cage system used in our latest published study (Zocher et al., 2020) provided the ultimate balance between resolution (animals can be localized to a single standard cage-sized unit) and accuracy (the rate of misreads is extremely low and appears to be limited to interference from a large group of animals in close proximity to the cage wall – as occasionally observed in sleeping clusters).

The optimized cage system is now produced and distributed as "Colony Rack" by PhenoSys, Berlin (Germany). It uses a double sided 5 × 7 rack for 70 conventional cages Eurostandard Type II (Tecniplast 1246C). Neighboring cages are connected horizontally and between levels by polycarbonate tubes. Connecting tubes pass through the circular antenna of an RFID reader. Readers on each of the 7 levels connect the total of 110 RFID readers via ethernet to a PC positioned on top of the rack.

The first publication with the new system (Zocher et al., 2020) confirmed the findings from our original study: genetically identical mice showed increasingly different and stable behavioral trajectories, which correlated with levels of adult neurogenesis. In addition, the new study revealed that some of these induced individualizing changes are lasting, even after discontinuation of the exposure to the enriched environment and that among these changes are epigenetic modifications on genes that are involved in hippocampal plasticity.

7. General observations

This paragraph summarizes a couple of common, not experiment-specific observations that can be made, when mice are exposed to the paradigm. They show that mice readily adapt to the new multi-cage environment and use its features extensively. The mice explore the full area of the cage very quickly and consistently over time. They are well distributed, but prefer the company of others with typically four animals per cage (interestingly, this is the recommended capacity for the cages used). A consistent level of activity was recorded during the active nighttime periods. The mice mostly sleep in any of the cages with nesting material or houses provided or in those that have a food and water rack. If they do not find the nesting material in what appears to be their preferred location, they drag it to their preferred spot and thus sometimes fill the cages with large amounts of nesting material. Moving larger objects was also attempted but was obviously unsuccessful due to the constraints of the inter-cage tunnels. In addition, individual cages were often given assigned functions by the mice, with certain cages or cage sections preferentially used as sleeping places or toilets. With the larger experiments, we have observed fragmentation of the social group into sleeping clusters (limited by the physical constraints of the cages and bedding). This social clustering does not appear to persist in interactions during the active nighttime period, however. Food and water provided ad libitum in a subset of cages was readily accessed by all animals and no nutritional or social issues were detected in any of our experiments to date. Although social problems did not occur, the ENR did result in less socially exploratory behavior, such as naso-nasal sniffing and approaching, compared to standard mice (as observed visually). Mice tend to spend more time in the compartments closest to food and water and less rarely explore the upper compartments. As described for conventional enriched environments, mice living in the individuality cage were lighter than age-matched controls, an effect that disappeared once enrichment was discontinued (Zocher et al., 2020). When tested for object recognition, over the course of two trials, enriched mice showed higher object exploration in both trials in comparison to animals living under standard conditions (Zocher et al., 2020). Moreover, 6 months of shared environment in the individuality cage led to higher repeatability of object exploration (> 0.5 repeatability indicates that most of the variation is due to interindividual differences rather than within-individual differences).

Individually tracking animals within an environment revealed an increased interindividual component of variance throughout weeks and months, despite controlling for genetic and environmental factors (Zocher et al., 2020). As mentioned above, behavioral stability over time, an important aspect of individuality, cannot be investigated by cross-sectional study designs. Data collected from the individuality cage not only exposes individual differences in behavior (behavioral variation), but also reveals within-individual behavioral consistency over time.

8. Data acquisition and handling

The data provided by the system represent a continuous documentation of behavioral activity but they differ from a record as it would be provided by video monitoring. A data record entry here includes the sensor ID, the sensor XYZ location, the animal ID, the time of detection onset, the duration of the detection interval, and the number of times the

tag ID was transferred to the sensor during the detection interval. These data are saved to a file (comma-delimited text; CSV) which can be retrieved at any time during the experiment. At around 800 KB / mouse / day, data volumes (c. 5 GB for a typical 3-month experiment with 80 animals) are manageable with standard computer hardware.

For analysis, the raw data need to be converted into animal-specific trajectories through the cage system. Several methods exist for this and a software package is in preparation at the time of writing. Here, a brief overview of several alternative approaches will be given. Note that different approaches to the data will provide access to different types of questions and that the data sets can be reanalyzed later with new methods. Some general principles, though, help to understand the particular properties of the paradigm and hence the structure of the data.

The location of the animal, for example, can only be determined at the time of antenna contact, and its position at other times is inferred from the temporally adjacent contact data. One approach is to infer a trajectory by linking up contacts and mapping this to the physical cage layout. Another approach is to consider contacts that span a cage as evidence for presence in that cage. In either case, the cage network can be thought of as a graph (in graph theory terms) with cages linked by tunnels; allowing measures such as shortest paths and cage centrality to be calculated. It is also possible to include real distance measurements in 3-dimensional Euclidean space to obtain parameterized measures of movement speed.

In order to estimate the trajectory of an animal in the cage system, it is important to obtain sufficient data to interpolate misread data points. Uncertainty about the animal's position can affect many of the possible read-out values from the system. Although Roaming Entropy (RE, see below) is relatively robust to fragmented trajectory information, other potentially interesting measures, such as movement speed or contact time with other animals, can be more profoundly affected by poor data quality. In particular, it can be impossible to unequivocally interpolate a path where multiple alternative trajectories exist (i.e. multiple shortest paths if the cage network is viewed in terms of graph theory) and this problem worsens with an increased number of antenna misreads. The current 'ColonyRack' cage design with spatially isolated low-power ring antennae on connecting tunnels has all but removed the problem of missing data, allowing us to explore more detailed analysis of animal trajectories.

A flexible method that is currently being explored would consist of expanding the raw contact data into a high-resolution (typically using 5 s intervals) cage presence matrix, where the location of each animal during each 5-s interval is determined. This intermediate data format can be further processed to perform feature extraction, which in turn would result in a feature matrix (including measures such as animal speed, roaming entropy etc.) for each animal at each time point. While initial pre-processing of these data is relatively processor- and memory-intensive (around 30 min utilizing 24 parallel threads and c. 60 GB RAM for 80 animals over a 3-month experiment), the resulting output data are small (c. 20 MB) and can be easily analyzed on a standard laptop. Of the many parameters that can be extracted, initial work has focused on 'roaming entropy' as an easily-calculated measure of exploration within the multi-cage environment.

9. Roaming entropy

As an etiological valid index of exploratory behavior in mice, we have introduced roaming entropy (RE). RE is a measure of the predictability of the whereabouts of an animal in a given period of time. RE implements the assessment of mobility and diversity of experience with a measure of territorial coverage and exploration. For teaching purposes, we have developed an online tool that computes RE on-the-fly as one moves the mouse pointer (no pun intended) over a virtual cage (https://www.brandmaier.de/roamingentropy/). While readers might skip the following paragraphs, the details about RE might help to see the further (including translational) potential of the paradigm and the type of data it delivers.

RE was inspired by information-theoretic accounts of predictability and entropy (Shannon, 1948). Shannon entropy is the average level of surprise or uncertainty in the possible outcomes of a random variable. RE is computed as the entropy of the empirical distribution of places an animal has visited over a given period of time:

$$RE = -\left(\sum_{i=1}^{n} p_i log\, p_i\right)$$

with p_i being the probability that location i was visited by an animal (that is, the proportion of a given period of time spent in each unique location), and n is the total number of unique locations in the environment. To compute RE, we first discretize the antenna contacts over a given period of time (e.g. a given night) into blocks and record the last antenna contact for that block. For example, when we discretize into blocks of 5 s and investigate a single night of 12 h, we obtain a discrete time series of 8640 blocks. Then, we compute the distribution over locations from the observed location frequencies over these blocks. RE reaches its maximum when the probability of being at a given location is equal across all locations (uniform distribution). The maximum RE is log (n). To standardize RE, we usually compute normalized RE (NRE), which ranges between 0 and 1:

$$NRE = -\left(\sum_{i=1}^{n} p_i log\, p_i\right) \Big/ log n$$

(N)RE is low when a mouse has a stable and small home range. But even a relatively large range can be covered with low RE if few stable locations dominate the observed antenna contacts. (N)RE is high if the animal visits many locations, each for a roughly equal amount of time – however, the dwell times in one location must not necessarily be contiguous. For example, assume there were only two antennas in a cage, one in the home cage and one at a food source. If a mouse spent 75% of the time in its home cage and 25% at a food source, the resulting entropy would be $-(0.95 \log 0.95 + 0.05 \log 0.05) = 0.20$ and NRE = 0.29. If it spent equal amounts of time at both places, the RE would be $-(0.5 \log 0.5 + 0.5 \log 0.5) \approx 0.693$ and NRE = 1.

RE is particularly useful if sensor placement is sparse or unequal (e.g. when sensors are placed at points-of-interest rather than on a grid) because measures like total path length or velocity cannot be computed; however, note that absolute RE values are not necessarily directly comparable across different antenna setups.

Under the assumption that unpredictability of behavior corresponds to a greater novelty of experience, we have investigated cumulative roaming entropy as the cumulative sum of NRE (CRE) values across multiple weeks. In our previous research, we have found associations of individual trajectories of CRE with patterns of social behavior and structural brain plasticity (Freund et al., 2015). We have used latent growth curve models to model changes of (C)RE (Freund et al., 2013) and assess increasing degrees of individualization. However, even if entropy over multiple time-segments is similar, the visited locations may be different. For example, if two out of twenty locations are visited at equal proportions of time, RE is identical regardless of which of the two states is chosen. If we want to make statements about the dissimilarity of two distributions of antenna contacts, we can compute their *relative entropy* or *mutual information*. This is useful for either assessing differences in behavior of a given animal across different points, or between animals in the same period of time (e.g. (Shemesh et al., 2013) or for clustering together similar behaviors based on complexity (also see (Brandmaier, 2015)).

Roaming entropy has also been successfully used in human studies of individual differences in behavior. For example, Saeb et al. found that roaming entropy of geolocation data recorded from mobile phones correlated with depression symptom severity (Saeb et al., 2015). Heller

et al. found further support that RE is associated with greater experiential diversity as RE was significantly correlated with entropy over a sociodemographic feature space of the environment (Heller et al., 2020). Further, they showed that daily variability in physical location was associated with increased positive affect in humans.

10. Other readouts from the cages system

Beyond roaming entropy, the time stamped individual position data allows deriving additional behavioral parameters that inform about physical fitness, about sociality, hierarchical status, about behavioral stereotypy and give an index of anxiety vs. exploratory drive. Initial analysis isolates the 24 h tours that each individual moved through the cage system. The total distance moved in 24 h provides a parameter of physical activity. Tour data can be subdivided into segments of equal horizontal travel distance and then allow comparing maximum travel speeds over such standardized distances between individuals. This can also be done specifically for the up and down movement between levels which is somewhat equivalent to the physical exercise of running up and down a staircase. Established methods, as also used for wheel running data, exist to extract parameters of circadian rhythmicity from 24 h activity data collected over weeks or months. When an animal leaves a cage through a tube different from the original entry tube one can deduce the animal was present in that cage during the time interval between entry and exit. This allows determining which animals spent time together in a cage both during the active phase and during the resting phase. The large number of such observations obtained from weeks of recording in the colony cage allows determining social network structure and its social dynamics over time. Similarly, times of presence in the feeding cage can be individually determined. This provides an index of social rank since low ranking individuals typically avoid crowding and feed more at the less preferred feeding times. If one takes 24 h tours and then compresses the data using an algorithm such as ZIP the compression ratio will be higher if the data contains more repetitive sequences. Being exposed to fresh bedding lacking the odor profile of the formerly used bedding is a strong novelty stimulus to mice. Individuals react differently to a novel environment as a consequence of their individual balance between anxiety and exploratory drive. This can be individually assessed after the weekly bedding changes. Taken together, the time stamped position data from the individuality paradigm provide a rich source of information for characterizing multiple dimensions of behavioral individuality.

11. Addressing behavioral variability in cross-sectional designs

In addition to longitudinal home cage tracking, behavioral variability can also be assessed using traditional behavioral tests by comparing variance differences in task performance between two or more animal groups in a cross-sectional study design. For instance, we have previously shown that the open field test and novel object exploration test can be used to detect the inter-individual differences in exploratory behavior that mice develop when housed in one large enriched environment. Specifically, we found that enriched housed mice showed increased variances in spatial exploration (roaming entropy) in an open field arena and in the exploration of objects placed into the arena compared to mice that were housed in control cages (Körholz et al., 2018; Zocher et al., 2020). While it can be laborious to test the large numbers of mice required to reliably detect variance differences, an advantage of this approach lies in the controlled conditions of traditional behavioral tests, and in the ability to more easily dissect different aspects of behavior, such as separating spatial exploration from social interactions. Moreover, a plethora of cognitive tests exist for which the specific cognitive processes and relevant brain areas are well-characterized, allowing for cross-sectional testing of cognitive variability. Performing repeated behavioral testing can even yield information about the stability of the analyzed traits over time. For instance, using novel object exploration tests at two periods of time, we demonstrated that the individual differences in object exploration that mice develop in an enriched environment persisted within individuals even after they were withdrawn from the enriched environment for three months (Zocher et al., 2020). While cross-sectional behavioral testing enables assessing behavioral variability at defined time points, due to the low temporal resolution and increased stress levels during animal handling, it does not allow investigations of developmental trajectories of behavioral individuality. Combining cross-sectional analysis of mice in well-characterized behavioral tests with longitudinal home cage tracking can, however, yield complementing insight and can inform analysis and interpretation of the longitudinal mouse activity data.

12. Other home-cage systems to study individuality

Our Individuality paradigm is not the only experimental attempt to more systematically address individuality in rodent populations under highly defined conditions.

The fine grained analysis of social structures in laboratory colonies of mice was the subject of a study by Shemesh and colleagues (Shemesh et al., 2013). Individuality was not explicitly subject of that study, but within-group differences in behavior and the emergence of high-order group structures that depend on the different interactions between the mice in the cage. The paradigm compared 17 groups of mice in one 0.35 m^2 cage, equipped with toys, walls, ramps and nests. Groups of 16 mice were followed with a video system and color marking of the fur with fluorescent dyes. The analysis side of this approach was later developed further to identify stable patterns of social behaviors in the groups of mice, resembling 'personality' traits (Forkosh et al., 2019). Arguably, the term personality, which is to some extent controversial in the animal literature, goes even one step further than 'individuality'. But Forkosh et al. define "personality [as] a complex entity that reflects stable individual differences and, in so doing, maps the space of phenotypic variability", which is close to the operational definitions of individuality that we derive from our experiments. By means of optogenetically manipulating oxytocin-expressing neurons in vivo, the development of patterns of social behavior could be altered extrinsically, further extending the reach of this approach (Anpilov et al., 2020).

'*Souris city*' by Philippe Fauré and colleagues is another sophisticated tool to study groups of usually 10 mice in a an enriched environment with respect to their emerging individuation of social behavior (Torquet et al., 2018). The group could show that the development of individual behavioral patterns was reflected in differences in activity in midbrain dopaminergic neurons (Torquet et al., 2018). One key difference to our approach is the smaller number of mice to be studied and (to date) the much shorter experimental periods.

In addition, a number of protocols, based on video, RFID or a combination of both have been published that allow to address social behavior in mice cohorts longitudinally, but have not yet provided in-depth analyses with these approaches (Howerton et al., 2012; Ohayon et al., 2013; Peleh et al., 2019; Puścian et al., 2016; Weissbrod et al., 2013). These publications also have not made explicit reference to the question of individuality. So far they have all been used for short-term studies (sometimes as short as 24 h only) and not up to 6 months as in the case of our paradigm. Although the approaches might be theoretically suited to delineate trajectories of social behavior, this remains to be shown in practice.

13. The non-shared environment and the future of environmental enrichment

The Individuality paradigm moves beyond the conventional enriched environment, because it is longitudinal and focuses on the between-animal differences. While it can be combined with cross-sectional between-group comparisons, its key asset is that it makes individual longitudinal behavioral patterns assessable. Because the

enriched environment itself is shared by all mice in the cage, the classical take of the paradigm as allowing to decipher the environmental contribution to phenotypic variation (if the genetic background is controlled), is not applicable here. In our model, both genes and environment are kept constant. The emerging variability and, hence, individuality, is equivalent to the so-called "non-shared environment" (Plomin and Daniels, 2011; Turkheimer and Waldron, 2000). The non-shared environment is the part of the non-genetic effect that is related to the behavior of the individual and tends to increase differences rather than homogenize them. The concept has been introduced by Robert Plomin, who had asked why siblings in the same family are so different from one another (Plomin and Daniels, 2011). Despite its high face validity, the non-shared environment has been experimentally elusive. We now propose that the Individuality paradigm is a tool to study the non-shared environment in mice (Freund et al., 2013; Kempermann, 2019).

In addition, however, with their behavior, mice also shape the shared environment of the other mice in the cage: the non-shared environment spills over into the shared environment in that individuals within the mouse population alter the environment. The size of this effect cannot easily be estimated but represents an important facet in attempts to translate the results from the paradigm to human conditions. Our ability to control genotype in this context greatly improves the accessibility of the non-genetic component in this situation (Scarr and McCartney, 1983).

Aspects of the Individuality paradigm can be incorporated into classical environmental enrichment experiments. Using equal-sized control and enriched groups, for example, allows to reliably compare variance between the groups (Körholz et al., 2018). While variance is not equivalent to individuality, it provides an important first indication. Overall variability is composed of intra-individual (temporal) variation and inter-individual variability of emerging stable traits. Whereas individuality is usually interpreted as a stable property, the question remains to be resolved, to which extent instability of behaviors over time also represents an individualizing feature. In theory, this analysis might become achievable with the Individuality paradigm.

Our findings indicate that the venerable paradigm of enriched environments still faces exciting times ahead and can be further developed to experimentally address non-genetic sources of variation in biomedical contexts. Through these investigations, we can further understand the underlying mechanisms of individuality development and thus possibly also establish approaches for individual therapy concepts for patients. The system can be used with genetic models of disease, as long as mutant and control mice can be held together within one cage (which has to be tested before). It has to be kept in mind for such studies, though, that different genotypes means indirect effects on the shared environment, creating a source of between-group or between-animal variation.

In our own research we relate the paradigm to the question of how individual behaviors form neural reserves to cope with the impediments of the aging brain and the consequences of neurodegeneration. As such, we believe that the Individuality paradigm holds the promise to provide one of the few experimental approaches to understanding at a fundamental neurobiological level, how individual behavior can actually contribute to support healthy cognitive aging, built reserves and shape and maintain resilience.

Declaration of Competing Interest

YW is owner of PhenoSys, the manufacturer of the cage system presented in this article. The other authors declare no conflict of interest.

Data availability

No primary data were used for the research described in the article.

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