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ASSOCIATION BETWEEN EXPLORATORY ACTIVITY AND SOCIAL INDIVIDUALITY IN GENETICALLY IDENTICAL MICE LIVING IN THE SAME ENRICHED ENVIRONMENT

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Abstract—We previously reported that inbred, genetically identical mice living in one enriched environment develop individual behavioral trajectories, indicating increasingly different levels of spatial exploratory behavior as quantified by roaming entropy. Cumulative roaming entropy (cRE) correlated positively with adult hippocampal neurogenesis, a type of plasticity involved in the flexible integration of new information into existing contexts (Freund et al., 2013). The study on which we report here was done in parallel to that first experiment, but here we acquired detailed observational data on the behavior of individual mice. Roaming entropy (RE) was again assessed in real-time with an antenna-based system over the entire experimental period of 3 months. Compared to the least active mice in the enclosure (low number of antenna contacts), the most active animals showed tendencies of increased socially interactive behavior in the final observation block whereas least active mice displayed more self-related behavior (non-social local exploration and play). When looking at roaming behavior, we discovered that RE correlated negatively with latent factors representing social exploratory and non-social exploratory and play behavior. Adult neurogenesis could not be studied in the present cohort but we do know that under identical conditions, cumulative RE correlated positively with adult hippocampal neurogenesis. We can thus hypothesize that the mice with more exploratory experience in terms of areal coverage (as quantified by RE) and related greater levels of adult hippocampal plasticity, might also be the ones that were less involved in interactions within the group and, hence, more individualistic. While this remains to be confirmed experimentally, the present data suggest that the described mechanism of individualization, which has previously been shown to be hippocampus-dependent, has a social component.

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Key words: hippocampus, dentate gyrus, experience, adult neurogenesis, plasticity, exploration.

INTRODUCTION

Shemesh and colleagues have reported that mice exposed to an enriched environment during adolescence became more individualistic than under control conditions, in the sense of a weaker dependency between the behavior of individual mice (Shemesh et al., 2013). They drew their conclusion from a detailed analysis of social interactions among the mice based on joint spatial configurations derived from a video-based tracking system. In their discussion, Shemesh et al. highlighted the suggestive relation of their data to our finding that genetically identical mice sharing one enriched environment for a period of 3 months developed stable behavioral trajectories that correlated with adult hippocampal neurogenesis as a measure of structural brain plasticity (Freund et al., 2013).

In the original set-up for the study previously reported by us (Freund et al., 2013), we paralleled two identical experiments, (sharing one control group) the only difference being, that in one enclosure we assessed histological measures of adult neurogenesis at the end of the study, and in the second enclosure we performed a detailed behavioral monitoring. The aim of the behavioral monitoring had been to gain deeper insights into behavioral patterns beyond those assessable by the automated tracking system, for instance, signs of aggressiveness, play behavior, maintenance behavior, and social interaction. We planned to complement the behavioral

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Abbreviations: cRE, cumulative roaming entropy; CTR, control group; ENR, enriched environment; LA, least active; MA, most active; RE, roaming entropy; RFID, radio-frequency identification.

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monitoring data with an immunohistochemical follow–up to replicate and broaden our statements about brain–behavior relations; unfortunately, fixation of the mouse brains in the second enclosure failed and all histological data were lost. Although this prevents us from making strong statements about the relationship between more fine-grained behavioral patterns and adult neurogenesis, we do have available the automated tracking data for both environments. From the results in the first enclosure, we also know about the strong correlation between cumulative roaming entropy (cRE) and adult neurogenesis, obtained from the same experimental setup (in particular identical cage layouts, maintenance and handling routines as well as the same staff), the same delivery of animals, and at the same time. We thus analyze the behavioral data set against the backdrop of the neurogenesis data that have already been published (Freund et al., 2013). Based on the data from the second enclosure, we asked the question of how far a large and complex environment would shape individual behavioral profiles in genetically identical mice. Specifically, we intended to learn whether any arising differences in observed behavior would be related to particular RE patterns. We would take any such correlation as the basis for cautious inferences on the relationship between various aspects of behavior and hippocampal neurogenesis, which need to be confirmed eventually in a follow-up study.

EXPERIMENTAL PROCEDURES

Animals and experimental groups

We purchased 100 female C57BL/6N mice from as many different litters as possible from a commercial breeder (Charles River, Sulzbach, Germany) at 4 weeks of age; 52 of these were used for the present study. During the first week in our lab, the mice were kept in groups of 10 (see Fig. 1 for the experimental set-up). Within this week, all animals had a radio frequency identification transponder (RFID; Trovan ID-100B Animal Implantable Transponder) implanted in their necks under brief anesthesia with isoflurane. Using marker pens, the mice were marked on ears and tail with an individual color code in order to make them visually distinguishable. The markings were renewed once a week during cage maintenance. The animals were randomly distributed to either the enrichment group (ENR; 40 mice) or the control group (CTR; 12 mice). The randomization was done using a freely accessible computer program for scientific randomizations at www.randomizer.org. In both groups, the animals had bedding, nesting material, and free access to food and water. The animals were kept in a light/dark cycle of 12 h per phase.

After 105 days in their respective enclosures, ENR and CTR animals were deeply anesthetized with Ketamine and Xylazine (Ketanest®, 100 mg/kg body weight and Rompun®, 10 mg/kg body weight in saline solution). This was followed by a transcardial perfusion with 0.9% NaCl and subsequently with 4% paraformaldehyde (Roti®-Histofix 4%, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). The brains were dissected below the brainstem and subsequently wet-weighed. Three weeks before, all animals received three injections of BrdU (5-bromo-2′-deoxyuridine; SIGMA–Aldrich; conc.: 50 mg/kg body weight) intraperitoneally, one on each of three consecutive days. Because of a fixation failure, antigens were not appropriately preserved in the tissue samples and cell genesis could not be further explored in this study.

In all experimental steps, we strictly adhered to national laws and institutional guidelines, and all experiments were approved in advance by the appropriate authorities at Westfälische Wilhelms-Universität Münster (Reference No. 8.87-50.10.36.08.250).

Enclosures and tracking system

40 ENR animals lived in an enclosure with a square ground area of 1.75 m side length, 2 m height, and a total area of approximately 5 m^2. The enclosure's interior and positions of the antennas were identical to the enclosure we described before (Freund et al., 2013): The basic setup featured two ground levels and three elevated levels through which the animals could move freely. Water and food sources were available at each level. All levels were connected with plastic tubes. Two nesting boxes were provided and various enrichment objects were placed in the enclosure (e.g., plastic and cardboard tubes, wooden scaffold, flower pots). The mice could leave the enclosure, for example, in the event of high social pressure, by crossing a tube in the enclosure's front leading to a water basin. After crossing the water, they could enter an emigration cage.

Data were collected using the software program Jerry 2 Recorder, developed for these purposes by the Institute for Geoinformatics in Münster, and stored using a MySQL database (Kritzler et al., 2006).

Twenty plastic rings with RFID antennas were installed throughout the enrichment enclosure, recording

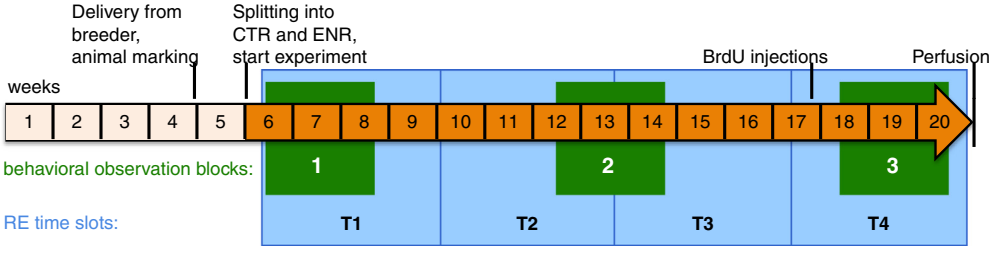

Fig. 1. Experimental schedule (see text for details).

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