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Loss of individualized behavioral trajectories in adult neurogenesis-deficient cyclin D2 knockout mice

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Abstract

There is still limited mechanistic insight into how the interaction of individuals with their environment results in the emergence of individuality in behavior and brain structure. Nevertheless, the idea that personal activity shapes the brain is implicit in strategies for healthy cognitive aging as well as in the idea that individuality is reflected in the brain's connectome. We have shown that even isogenic mice kept in a shared enriched environment (ENR) developed divergent and stable social and exploratory trajectories. As these trajectories—measured as roaming entropy (RE)—positively correlated with adult hippocampal neurogenesis, we hypothesized that a feedback between behavioral activity and adult hippocampal neurogenesis might be a causal factor in brain individualization. We used cyclin D2 knockout mice with constitutively extremely low levels of adult hippocampal neurogenesis and their wild-type littermates. We housed them for 3 months in a novel ENR paradigm, consisting of 70 connected cages equipped with radio frequency identification antennae for longitudinal tracking. Cognitive performance was evaluated in the Morris Water Maze task (MWM). With immunohistochemistry we confirmed that adult neurogenesis correlated with RE in both genotypes and that D2 knockout mice had the expected impaired performance in the reversal phase of the MWM. But whereas the wild-type animals developed stable exploratory trajectories with increasing variance, correlating with adult neurogenesis, this individualizing phenotype was absent in D2 knockout mice. Here the behaviors started out more random and revealed less habituation and low variance. Together, these findings suggest that adult neurogenesis contributes to experience-dependent brain individualization.

KEYWORDS
hippocampus, home cage monitoring, learning and memory, plasticity, stem cell

Over decades and for most part, experimental biology has been focusing on describing group differences and has gone great length to maximize standardization in order to optimize effect sizes. From a translational perspective, however, within-group differences, their origins and mechanisms are increasingly moving into the center of interest. Variability was even reframed as healthy noise counteracting the reproducibility crisis (Richter et al., 2009). In the context of the neurobiology of reserves and resilience (Perneczky et al., 2019; Stern

et al., 2019, 2020), the non-genetic sources of such variation and especially those that arise from the individual's own actions and behaviors are critically important. Lifestyle-dependent neuroplasticity plays a central role in concepts to support healthy cognitive aging, the building of neural reserves and the promotion of resilience. The actual mechanisms that can be ascribed to the behavioral component of reserve formation, however, are difficult to dissect in situations with great variance at the level of both genetic background and environment, which is the usual situation in humans (McQuail et al., 2020).

Using mice as model organism we have developed a novel experimental paradigm (Kempermann et al., 2022), which is a variation of the classical concept of "environmental enrichment" (Diamond, 1988; Mohammed et al., 2002; Nithianantharajah & Hannan, 2006) and allows to isolate the effect of the so-called "non-shared" environment (Kempermann, 2019). The non-shared environment is the component of the environmental factor in phenotypic variation that is ascribed to the individual's behavior (Plomin & Daniels, 2011; Turkheimer & Waldron, 2000). We found that even in genetically identical mice living in the same shared environment, individuality still emerges. Stable behavioral trajectories of activity formed and explained a substantial part of the variance in adult hippocampal neurogenesis (Freund et al., 2013, 2015; Zocher et al., 2020). As the current theory goes that adult-born neurons in the hippocampus optimize the adaptation of the hippocampal network to the type and level of cognitive challenges that an individual tends to experience and the flexible integration of novelty into pre-established contexts (see summary in Kempermann, 2022), we hypothesized that adult hippocampal neurogenesis might be causal for the behavioral response to the (shared) environment. To test this hypothesis we set out to study the emergence of individuality in the near-absence of adult neurogenesis.

To do so, the experimental model had to meet certain criteria: adult neurogenesis had to be stably suppressed in a dentate gyrus that is otherwise as normal as possible. There should not be side-effects of the suppression in terms of inflammation or secondary neuronal cell death, which excluded irradiation to suppress stem cell function in the dentate gyrus (Han et al., 2016). There also should not be a recovery or even rebound of neurogenesis during the experimental period of more than 3 months. The latter criterion excluded all models that chemically or genetically ablate neurogenesis. The regulatable genetic models are also excluded by the impossibility to give drugs like ganciclovir or doxycycline, which act as inducing ligands in these models, for the required extended periods of time. Consequently from the large variety of methods that have been used to experimentally suppress adult neurogenesis in rodents, we settled for constitutive cyclin D2 (Ccnd2) knockout mice (D2 k.o.), because of its stability and independence of an intervention by the experimenter during the course of the actual experiment.

For still unknown reasons, the cell cycle control in hippocampal neural stem cells of adult (but not developing) mice postnatally looses the redundancy in its dependency on different cyclin molecules, so that adult D2 k.o. mice have a normally developed dentate gyrus but very low (though interestingly not fully absent) levels of adult neurogenesis (Kowalczyk et al., 2004). There was an unexpectedly linear dosage effect in that heterozygotes phenotypically fall halfway between wild-types and homozygous knockouts (Garthe et al., 2014). These mice were originally described as having no neurobehavioral phenotype (Filipkowski & Kaczmarek, 2018; Jaholkowski et al., 2009; Urbach et al., 2013), but we could show that while learning itself was unimpaired, those aspects of learning that have in other models been ascribed to adult neurogenesis were decreased: flexibility was reduced and the mice showed a less efficient progression to hippocampus-dependent learning strategies (Garthe et al., 2014). We and others have extensively characterized these mice (see below for further discussion of the phenotype).

The concrete hypothesis of the present study was that adult-born neurons through their plastic effects on navigating the environment are key drivers of individuality. We followed the following specific aims: (1) Do the wildtype mice that share the cage with the D2 k.o. mice develop the enrichment phenotypes on behavioral variance and adult neurogenesis that we reported previously (Körholz et al., 2018)? (2) Is there evidence of confounding metabolic side-effects of the D2 mutation? (3) Do the D2 k.o. mice in the Individuality paradigm show the behavioral consequences of suppressed adult neurogenesis and is there an interaction effect by enrichment (Garthe et al., 2016)? (4) What are the effects of the D2 mutation and suppressed adult neurogenesis on the emergence of stable individualizing behavioral trajectories?

1 | MATERIALS AND METHODS

1.1 | Animals and housing conditions

Cyclin D2 knockout mice had originally been obtained from the Nencki Institute, Warszaw, Poland, and have been breed at our facility since 2015. A total of 80 female animals, knockouts and littermate controls were kept at the Center for Regenerative Therapies (CRTD), Dresden, Germany, with food and water provided ad libitum and on a 12 h light/dark cycle. Control and enriched animals received the same fortified chow (no. V1534; Sniff) with 9% of energy from fat, 24% from protein, and 67% from carbohydrates. All applicable EU and national regulations regarding animal studies were obeyed and the study protocol was approved by the local authority (Landesdirektion Sachsen) under TVV 58/2016 (File DD24-5131/354/63). At an age of 4 weeks, 50% of the mice of either genotype were randomly assigned to the two different housing conditions either living in custom-built Individuality enrichment enclosure (marketed as "PhenoSys ColonyRack Individuality 3.0" by PhenoSys GmbH, Berlin, Germany) and referred to here as enriched housing (ENR), or standard laboratory housing in type-II cages and four animals per cage (STD). The cage system has been described in detail elsewhere (Kempermann et al., 2022). Briefly, it consists of 70 regular type II cages that are mounted on a standard, double-sided stainless steel rack (Tecniplast 1246C) and connected by connector tubes. Two tubes each connect adjacent levels. All connecting tubes are equipped with ring antennas that that read the transponder IDs of the individual mice that pass through. Hubs at the 7 levels connect the total of 110 radio frequency identification (RFID) readers via ethernet to an external computer. The spatial resolution of the tracking equals the size of a standard type-II cage; the overall floor area is 2.74 m^2. Food and water were available only on the middle level but not the others and remained at the same location over the entire experimental period.

1.2 | Experimental design and time course

At 5 weeks of age, animals were randomly distributed to the two conditions, which they entered at 6 weeks of age. The experimental groups consisted of 40 female animals per genotype, housing 80 animals together in the same enriched environment and 80 animals under STD conditions (4 mice per cage) and separate for each genotype. Under brief isoflurane anesthesia ENR received an injection of a microtransponder (SID 102/A/2; Euro I.D.) under the skin of the neck. At this time also blood was collected from the retrobulbar venous plexus.

Every 2 weeks, all mice were removed from the cages, the toys and accessories changed in position and complexity and cages and tunnels were cleaned. Body weight was measured biweekly and glucose levels every 2 weeks in a drop of blood from the tail using Accu-Check test strips (PZN 6114963, Accu Check Aviva).

Four weeks before the end of the experiment, the mice received three intraperitoneal injections of BrdU (50 mg/kg, Sigma) 6 h apart on 1 day and were killed 28 days later with a mixture of ketamine/xylazine and a transcardial perfusion with 0.9% saline and 4% paraformaldehyde (PFA). Brains were left in 4% PFA over night at 4°C and were transferred to 30% sucrose before sectioning on a dry-ice cooled sliding microtome (Leica) at 40 µm thickness.

1.3 | Analysis of RFID data

Contacts of the mice to the RFID antennas in the tunnel were recorded using the software PhenoSoft Control (PhenoSys GmbH, Berlin, Germany), which saved antenna and mouse identifiers together with the time stamp of the antenna contact into a database. In total 84 days/nights were recorded. Raw data of the antenna contacts were binned into 5 s intervals for further processing as described before and aggregated into four time blocks for some of the analyses. The data and additional meta data are available at https://doi.org/10.5061/dryad.5hqbzkh9z. Noise reduction and calculation of RE were performed as previously described (Zocher et al., 2020). Because mice are night-active, only the events recorded during the dark phase were used. Frequencies of antenna in this time series were converted to probabilities $\rho_{i,j,t}$ of a mouse i being at an antenna j at a night t. Shannon entropy of the roaming distribution was calculated as $\mathrm{RE}_{i,t} = -\sum_{j=0}^{k} \left(\rho_{i,j,t} \log \rho_{i,j,t}\right) / \log(k)$, where k is the number of antenna and t is the day (Kempermann et al., 2022; Zocher et al., 2020). Dividing the entropy by $\log(k)$ scales the RE to the range between zero and one. Data from nights following events that could disturb patterns of exploration, such as cleaning of the cage or behavioral testing, were excluded. Cumulative RE was calculated by the addition of mean RE from the four time blocks. All analyses were performed in a blinded manner.

1.4 | Water maze

Spatial learning was assessed in the Morris Water Maze task, as described previously (Garthe et al., 2016; Garthe & Kempermann, 2013), but with an acquisition protocol of three trials per day for 6 days, followed by a 4-day reversal phase with the platform moved to a new position. The pool had a diameter of 2 m and the water was made opaque with non-toxic white paint. The mice were released from a starting point that changed every 2 days (marked with orange triangles in Figure 3a). Each trial lasted until the mouse had found the platform but was capped at 120 s. When mice did not find the platform, they were guided to the platform and could rest there for 15 s. The first trial on Day 7 was performed as probe trial for 60 s without the platform. Swim paths were recorded with Ethovision software (Noldus) and analyzed with our Rtrack package (Overall et al., 2020).

1.5 | Tissue preparation and immunohistochemistry

Tissue fixation and immunohistochemistry for the analysis of adult neurogenesis were performed as described previously (Körholz et al., 2018). Briefly, brains were cut into 40 µm coronal sections using a dry ice-cooled copper block on a sliding microtome (Leica, SM2000R). Sections were stored at 4°C in cryoprotectant solution (25% ethylene glycol and 25% glycerol in 0.1 M phosphate buffer).

For the detection of BrdU-positive cells, the peroxidase method was applied. Briefly, free-floating sections were incubated in 0.6% hydrogen peroxide for 30 min to quench endogenous peroxidase activity. For DNA denaturation, sections were incubated in pre-warmed 2.5 M hydrochloric acid for 30 min at 37°C, followed by extensive washes. Unspecific binding sites were blocked in tris-buffered saline (TBS) supplemented with 10% donkey serum (Jackson ImmunoResearch Labs) and 0.2% Triton X-100 (Carl Roth) for 1 h at room temperature. Primary antibodies were applied overnight at 4°C (monoclonal rat anti-BrdU 1:8000; ab6326, Abcam). Sections were incubated in biotinylated secondary antibodies (Jackson ImmunoResearch Labs) for 2 h at room temperature. Antibodies were diluted in TBS supplemented with 3% donkey serum and 0.2% Triton. Detection was performed using the Vectastain Elite ABC Reagent (9 µg/mL of each component: Vector Laboratories, Linaris) with diaminobenzidine and 0.04% nickel chloride as chromogen (0.075 mg/mL; Sigma-Aldrich). All washing steps were performed in TBS. Stained sections were mounted onto glass slides, cleared with Neo-Clear (Millipore), and cover-slipped using Neo-Mount (Millipore). BrdU^{+} cells were counted exhaustively on every 6th section along the entire rostro-caudal axis of the dentate gyrus using a bright-field microscope (Leica DM 750), omitting the cells in the uppermost focal plane, when focusing into the section (Kempermann et al., 2003).

1.6 | Statistics

All experiments were carried out with the experimenter blinded regarding the experimental group. Statistical analyses were done

using the statistical software Prism 9 (Graphpad) and R (Team R, 2014). In R, for normally distributed measures, we used Welsh's *t*-test to compare means and *F*-test to test for equality of variance between groups. For repeated measures (longitudinal data), a linear mixed regression was performed using the lmer function from the lme4 package (Bates et al., 2015), and *p* values were obtained by the likelihood ratio test of the full model against the model without the analyzed effects. Brown-Forsythe test from the car package was used to compare the variances between groups. Two-way ANOVA was applied to identify effects through housing and genotype using Prism and R. Data were visualized using violin plot function in Prism and the ggplot2 package in R (Wickham, 2011).

1.7 | Repeatability estimation

To assess stability of behavior, the components of variance and repeatability estimates were calculated for path length (cm) in the watermaze using generalized linear mixed models in a Bayesian framework, as previously described (Fong et al., 2010; Zocher et al., 2020). Repeatability (*R*) is the fraction of total variance that can be attributed to inter-individual differences, rather than within individual differences, calculated as

$$R = \frac{V_{(\text{ind})}}{V_{(\text{ind})} + V_{(\text{res})}}$$

where $V_{(\text{ind})}$ represents the inter-individual variances and $V_{(\text{res})}$ the residual, or within individual, variances (Dingemanse & Dochtermann, 2013). We used "days" as fixed effect and an interaction between day and individual mouse identifier as random effect. With a random effect, it is possible to estimate inter-individual variances for each day, while the residual variances were estimated separately. To fit the model, we applied Markov chain Monte Carlo estimation with Gibbs sampling (MCMCglmm R package; Hadfield, 2010). A Gaussian error distribution was assumed with weakly informative default priors for the fixed effect (days). An inverse Wishart distribution prior was selected for residual variances. From the posterior (co)variance distributions, we derived the estimates of repeatability and inter-individual correlations, using a mode of the posterior density.

2 | RESULTS

2.1 | The D2 wildtype animals confirm the previously described individuality phenotype

We first established that the chosen animal model shows a phenotype that is consistent with the literature and represents a true model of ablated adult hippocampal neurogenesis (Filipkowski & Kaczmarek, 2018; Kowalczyk et al., 2004; Urbach et al., 2013). As pre- and perinatal neurogenesis is unaffected, the mice have a dentate gyrus of normal gross morphology. Relatedly, there is a very low level of residual adult neurogenesis (in the range of 20–30 cells as opposed to hundreds in WT), which speaks to the fact that the overall niche structure is intact (Garthe et al., 2014).

In the current study, D2 knockout mice showed extremely low levels of adult hippocampal neurogenesis (indicated by the numbers of BrdU-positive cells in Figure 1a,b), as previously reported (Garthe et al., 2014).

Enrichment increased the number of BrdU-positive cells in the WT animals as predicted from the literature (Kempermann et al., 1997) but not in the mutants (Figure 1a,b). Notably, we also observed the previously described enhancing effect of environmental enrichment on the variance in adult hippocampal neurogenesis (number of BrdU-positive cells, Figure 1b) (Freund et al., 2013; Zocher et al., 2020). These effects were absent in the D2 k.o. mice (inset in Figure 1b; *t*-test, $t = -1.2004$, df = 74, $p = .2338$; *f*-test: $F = 0.97294$, num df = 37, denom df = 32, *p* value = .9298).

Roaming entropy (RE) is a straightforward measure of exploratory behavior, general activity and territorial coverage that can be derived from the antenna data in our cage system. Roughly speaking, it represents the probability that a mouse is found at a given location at a given time (for a more detailed description see Kempermann et al., 2022). Analogously to previous studies, we found a correlation between RE and adult hippocampal neurogenesis ($R^2 = 0.10$) in the WT mice of the present experiments. Remarkably, this effect could also be observed for the very low residual levels of adult neurogenesis that exist in the D2 knockout mice (Figure 1d).

Taken together, these observations confirm that the D2 model indeed reflects mice with constitutively suppressed adult hippocampal neurogenesis, but that the wildtype littermates develop the expected inter-individual differences in adult neurogenesis that correlate with individual behavior.

2.2 | Blood glucose levels in D2 k.o. mice are within the physiological range

D2 k.o. mice had a constitutively lower body weight than controls. Usually, animals in an enriched environment tend to be lighter than under standard housing (Körholz et al., 2018) and this was observed in the current study as well (two-way ANOVA, housing effect: $F_{(1,\ 1352)} = 183.46$, $p < .001$; Figure 2a). Weight gains over the weeks of the experiment were parallel in all four groups. Enrichment lowered body weight in both genotypes, and there was an interaction effect of genotype in housing ($p < .001$).

As cyclin D2 is expressed in islet cells in the pancreas (Georgia et al., 2010) and drives beta cell self-renewal (Tschen et al., 2017), we wanted to ascertain that D2 mice did not have a potentially confounding pancreatic phenotype. We observed mildly elevated blood glucose levels across the weeks of the experiment compared to controls under both housing conditions, but well within the physiological range (two-way ANOVA, genotype effect: $F_{(1,\ 1270)} = 78.129$, $p < .001$;

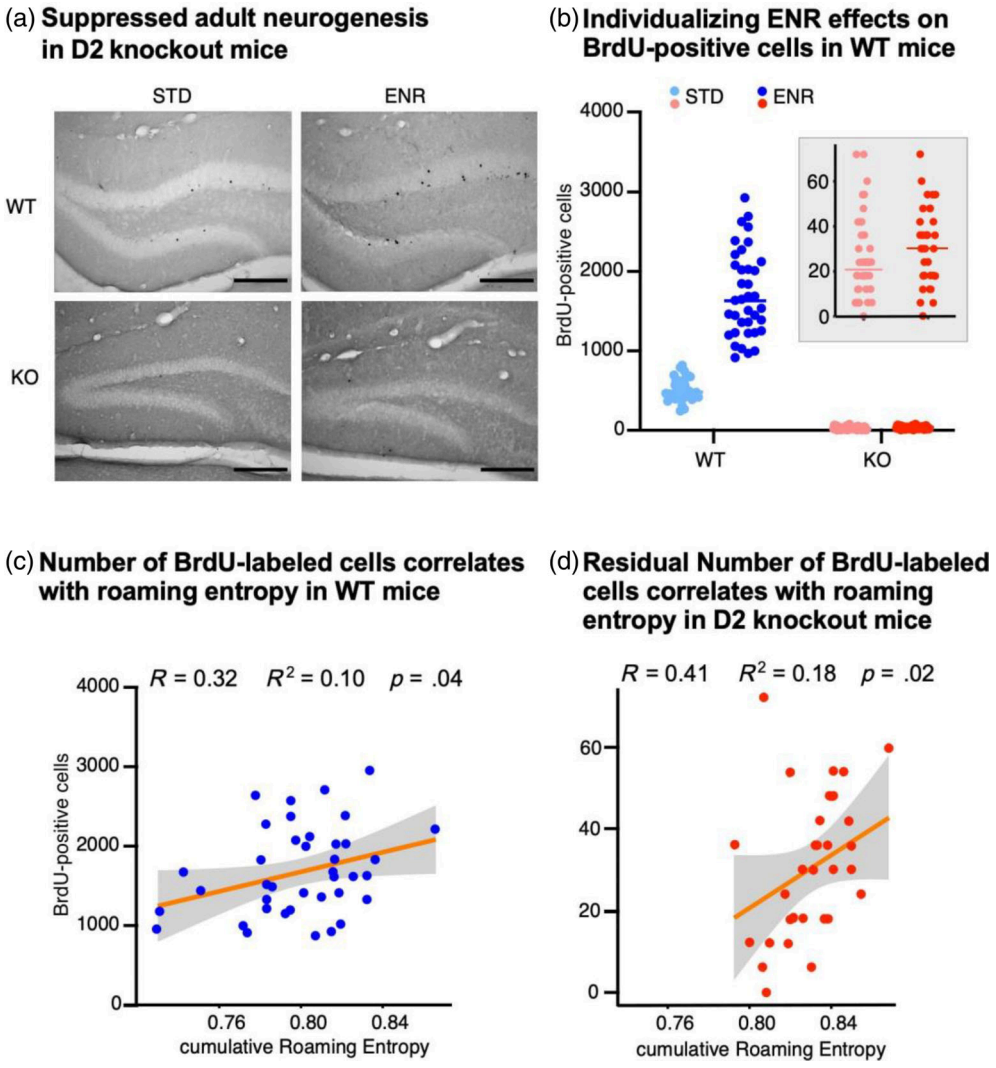

FIGURE 1 D2 knockout mice have suppressed adult hippocampal neurogenesis. (a, b) As previously described (Garthe et al., 2014), cyclin D2 knockout mice constitutively have very low levels of adult hippocampal neurogenesis compared to wildtypes. Adult neurogenesis was assessed by the number of BrdU-labeled cells 4 weeks after three injections of BrdU. (b), the low remaining numbers of new neurons are not increased by exposure to an enriched environment. In the wildtype animals, enrichment induced the previously described increase in mean and variance of adult neurogenesis (Körholz et al., 2018). (c) Under ENR conditions in the Individuality paradigm, there is a correlation between cumulative Roaming Entropy (cRE) as measure of exploratory activity and territorial coverage and adult neurogenesis in the wildtype animals, as observed in previous studies (Freund et al., 2013; Zocher et al., 2020). (d) Even for the by two order of magnitudes lower levels of adult neurogenesis in the D2 k.o. mice this relationship between cRE und the number of BrdU-positive cells is maintained. Further information on the statistical analysis is found in the main text.

Figure 2b). This is in accordance with other reports that D2 k.o. mice have normal insulin levels (Georgia et al., 2010).

2.3 | D2 k.o. mice show neurogenesis-dependent changes in the Morris water maze, which are not rescued by enrichment

We next investigated how D2 k.o. mice respond to environmental enrichment in terms of effects on hippocampus-dependent spatial learning. For this we used a protocol of the Morris water maze that we had previously identified as being sensitive to the functional contribution of adult neurogenesis (Garthe et al., 2009; Garthe & Kempermann, 2013). Adult neurogenesis allows the flexible integration of novel information into existing contexts, which is targeted by the reversal phase of this water maze protocol. In contrast to our previous studies, mice were here trained to criterion, which means that the acquisition phase ended only when all four groups had learned to task of navigating to the hidden escape platform in the pool (Figure 3a,b). The new data set confirmed that D2 k.o. mice learn the task. In the first days of training, there is a strong enrichment effect on the speed of acquisition, which is strongest in WT ENR animals, but this effect wears off. At the end of the acquisition phase there were no differences between the groups. All four groups also showed

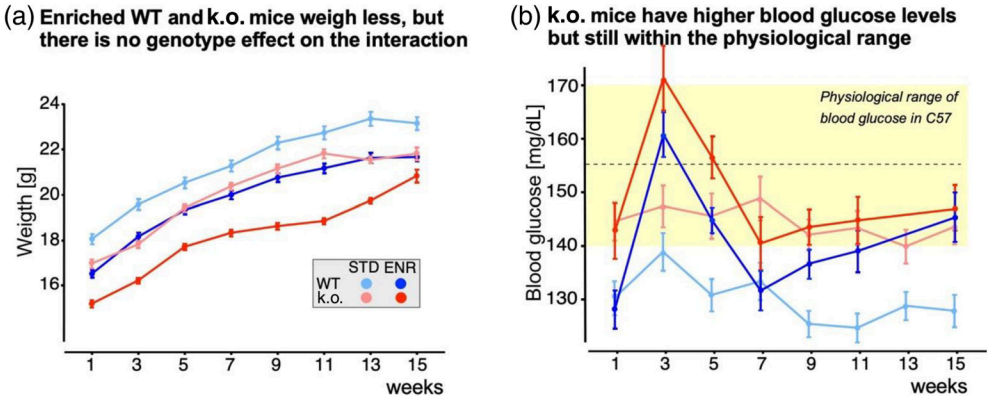

FIGURE 2 Body weight and glucose levels. (a) Over the experimental period there was the expected increase in body weight, parallel in all four experimental groups. As described previously, animals under ENR were lighter and there is an interaction effect of genotype and environment (statistical information in the main text). (b) Compared to wildtype mice under standard-housed conditions, the other groups had slightly elevated blood glucose levels, which however for all genotypes remained in the low physiological range.

robust learning in the probe trial on day 7, again supporting the notion that learning per se is unimpaired in D2 k.o. mice.

The characteristic effect of suppressed adult neurogenesis on reversal performance on strategy use and the impact of enrichment on strategy use was not as obvious in the current study than previously (Garthe et al., 2009, 2014, 2016; Garthe & Kempermann, 2013), which might be explained by the fact that in past experiments the mice had not been trained to criterion. However, when analyzing the learning strategies employed by the four groups of mice the expected differences became visible again, as previously reported (Figure 3c,d).

When learning the Morris water maze, mice go through a characteristic sequence of swim patterns that reflect different stages of the learning process (Garthe et al., 2009; Wolfer et al., 2001). Their search for the hidden platform becomes increasingly focused and only the advanced stages are hippocampus-dependent (see detailed explanation and discussion in Garthe & Kempermann, 2013). It could be shown that this hippocampus-dependent performance is reduced in mice lacking adult neurogenesis, especially under reversal conditions, when flexible relearning is required. This effect was also seen in the current study (Figure 3d,e). The proportion of spatial over non-spatial strategy usage was reduced in both standard and ENR conditions. D2 mice nevertheless showed ENR effects on WMZ learning, for example in that perseverance in the probe trial was reduced (Figure 3c).

2.4 | Behavioral trajectories of D2 k.o. mice show reduced individualization

The results presented in Figures 1–3 indicate that morphologically and behaviorally, D2 mice have the expected phenotype of suppressed adult neurogenesis, while still showing hippocampal learning including reversal learning—however at a characteristically reduced level.

Figure 4a reveals that the RE trajectories of D2 k.o. mice versus WT controls differ. Habituation (as apparent in the downward sloping trajectories) was reduced in D2 k.o. (Figure 4b; ANOVA: $F_{(1,\ 320)} = 25.299$, $p < .001$). The Pearson's correlation between mean RE values and night was $r = -0.64$ ($p < .0001$) in WT, but $r = .21$ ($p = .07$) in D2 k.o. That downward-sloping pattern in the WT is in accordance with our previous reports (Freund et al., 2013; Zocher et al., 2020).

At the same time, in WT, the standard deviation of RE, which is our key indication of increased individualization here, increased over time (Pearson's correlation, $r = 0.55$, $p < .0001$). In D2 k.o. mice, in contrast, there was no change in standard deviation across the experimental period ($r = -0.12$, $p = .33$; Figure 4c). The correlations between standard deviation and night were not equal between WT and D2 k.o. (Fisher's z, $z = 4.37$, $p = .00$) further supporting the genotype effect.

Note that the individual trajectories of Figure 4a and the group trajectory of Figure 4b are conceptually different: the latter describe a tendency in the group that is a consequence of the individual behaviors and their change over time. In our use of the term, a trajectory is simply a line of development that resembling the path of a moving physical body in space. From that perspective the "group trajectories" as in Figure 4b,c are indicative of specific changes in behavior at the individual level (as visualized in Figure 4a).

The conclusion is, thus, that in the absence of cyclin D2 the formation of individualizing behavioral trajectories was reduced. The D2 knockout mice remained more similar to each other than the WT animals in the same cage.

When binning activity data into four blocks and calculating how well activity in one bin predicted activity in the next, we found that the correlation between activities in the four blocks was reduced in D2 k.o. compared to WT. To compare RE correlation matrices, the sum of the squared Fisher transformed correlations, under the null hypothesis that the correlations are equal, were distributed as chi square. The matrices are significantly different further indicating that adult neurogenesis is necessary for long-term behavioral stability (Chi square = 16.25, df = 6, prob < .012, where prob = the probability of observing the Chi Square under the null hypothesis; Figure 4d). In D2

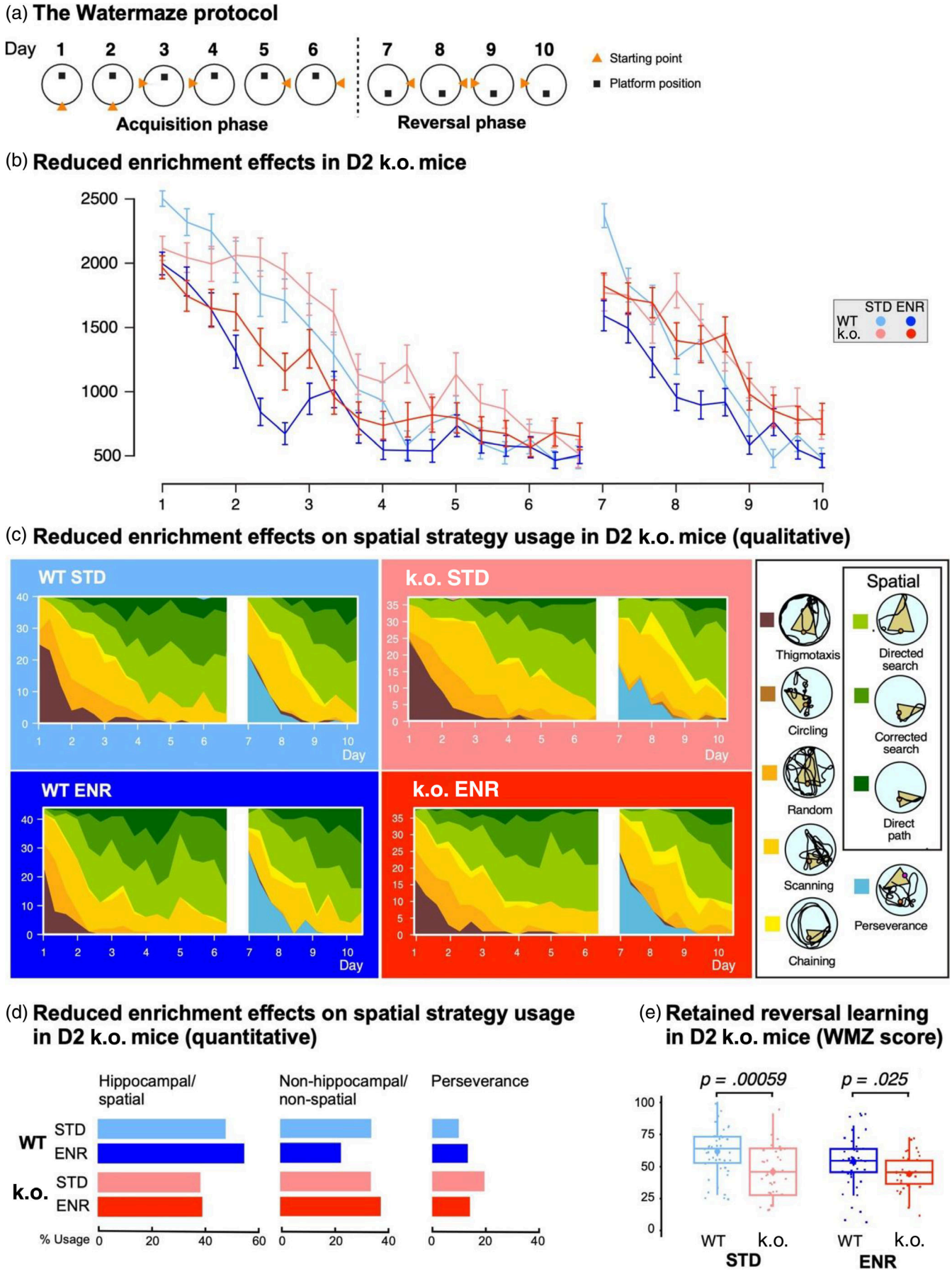

FIGURE 3 Legend on next page.

k.o. the activity trajectories were thus not only less diverging but also less stable in D2 k.o.

Analysis of the reversal phase of the watermaze beyond the results presented in Figure 3 focused on repeatability, that is the fraction of the total variance that is attributable to interindividual differences rather than intraindividual variation (Dingemanse & Dochtermann, 2013). For the entire reversal period this fraction dropped to very low interindividual contributions in standard-housed D2 k.o. mice. Enrichment rescued this decrease to some extent (Figure 5a). Figure 5b depicts an additional estimate of inter- versus intraindividual variance, "mean-scaled individual variation"—a standardized measure to estimate variation relative to the mean (Dochtermann & Royauté, 2019). Here, too, the interindividual variance estimate is diminished in standard-house wildtype animals, which rebounds under ENR conditions (dashed box in the lower panel).

These observations from the reversal phase indicate that some elements of individuality, which are sensitive to the suppression of adult neurogenesis under standard conditions, can still be compensated by neurogenesis-independent mechanisms that are stimulated by environmental enrichment (compare also Figure 3e).

3 | DISCUSSION

In this report we demonstrate that a constitutive knockout of the cyclin D2 gene in mice results not only in the functional and structural phenotype of reduced adult hippocampal neurogenesis, but also in a reduced emergence of inter-individual differences in the establishment of stable behavioral trajectories. We thus propose that the loss of adult neurogenesis, here concretely approximated by the number of BrdU-positive cells in the dentate gyrus at 4 weeks after injection of BrdU, is at least partly causal for the altered behavioral response and that, consequently, adult neurogenesis is required for the development of brain individuality.

Our key finding as reported in Figure 4, takes a reductionistic view on individuality. No unifying theory of individuality exists and there is as yet no defined neurobiology of individuality. We understand our results as small steps towards an emerging comprehensive concept, involving more elaborate models. In any case, individuality means (1) the establishment of an organismic entity, and (2) the distinction from others. Our research here focuses on the second aspect. We ask why phenotypic differences emerge, if genes and the shared environment are controlled. Hence, variance is a good first approximation of individuality in this context, but not identical to it.

Starting point of our investigation was the observation that in genetically identical mice in a shared environment inter-individual differences in behavioral trajectories emerged that explained up to 20% of the variance in adult hippocampal neurogenesis (Freund et al., 2013). A loss-of-function experiment testing the hypothesis that new neurons are required for the development of the described patterns in the RE trajectories appeared as the logical next step. The long experimental duration of 3 months and the lack of a possibility to monitor actual adult neurogenesis levels non-invasively in vivo, however, limited the range of possible experimental models. Pharmacological interventions, including those acting via genetic constructs (e.g., tamoxifen- or ganciclovir-based) are not practicable for long-term application: the monitoring and treatment of side effects would created massive confounds. Irradiation is associated with local inflammation (Monje et al., 2002). In any case, for most published models only limited information is available and few have been characterized in several studies (Ben Abdallah et al., 2013; Garthe et al., 2014; Petkova et al., 2020). We thus chose the D2 knockout mouse, which has been deeply phenotyped in multiple independent studies (Petkova et al., 2020). Even though the model is not free of potential confounds, it comes relatively close to the ideal. The neurogenesis-dependent behavioral phenotype of D2 k.o. mice is exceptionally well characterized (Ansorg et al., 2012) and—compared to other models of suppressed adult neurogenesis—an unprecedented range of other behavioral data exist for these mice (Filipkowski & Kaczmarek, 2018; Jaholkowski et al., 2009, 2011; Urbach et al., 2013). As in the neural stem cells of the hippocampus the redundancy of the cyclins ceases only postnatally, the mice have a normally developed dentate gyrus. The fact that there is a low residual level of adult neurogenesis is not fully understood but underscores that the neurogenic niche is functional.

A recent report also described reduced adult neurogenesis in the subventricular zone SVZ of the adult brain in D2 k.o. mice, resulting in reduced numbers of adult-generated interneurons in the olfactory bulb (Płatek et al., 2022). Potentially, such reduction in adult neurogenesis outside the hippocampus might contribute to effects on individuality. However, we know that the behavioral stimuli that work in the hippocampus have no effects on adult neurogenesis in the SVZ (Brown et al., 2003; Leiter et al., 2019). Most models of reduced adult hippocampal neurogenesis have not tested the SVZ and, in fact, most are not specific to the hippocampus, as the precursor cells share key

FIGURE 3 Effects of genotype and housing on water maze performance. (a) The water maze protocol consisted of a 6-day acquisition phase, followed by a 3-day reversal phase with a first trial on day 7 as a probe trial with the platform removed. The starting position (orange triangle) was moved after every 2 days. For the reversal phase, the platform was moved to a new position. See Garthe and Kempermann (2013) for details on the protocol and the underlying rationale. (b) Swim path in cm reveal good learning curves for all four groups, which reach the criterion approximately on day 4. As expected ENR WT mice have a steeper learning curve, the knockout mice take longer. All animals show reversal learning but efficiency was lower in D2 k.o. (c) Analysis of the swim patterns reveals difference in hippocampal/spatial strategy usage. (d) Under WT conditions, ENR increases the use of spatial strategies. In D2 k.o. the use of spatial strategies is reduced, which is not rescued by ENR (Garthe et al., 2016; Garthe & Kempermann, 2013). (e) Compared to WT, D2 k.o. mice consistently had a reduced ratio of hippocampal to total strategies during the reversal phase, but there was no effect of housing here.

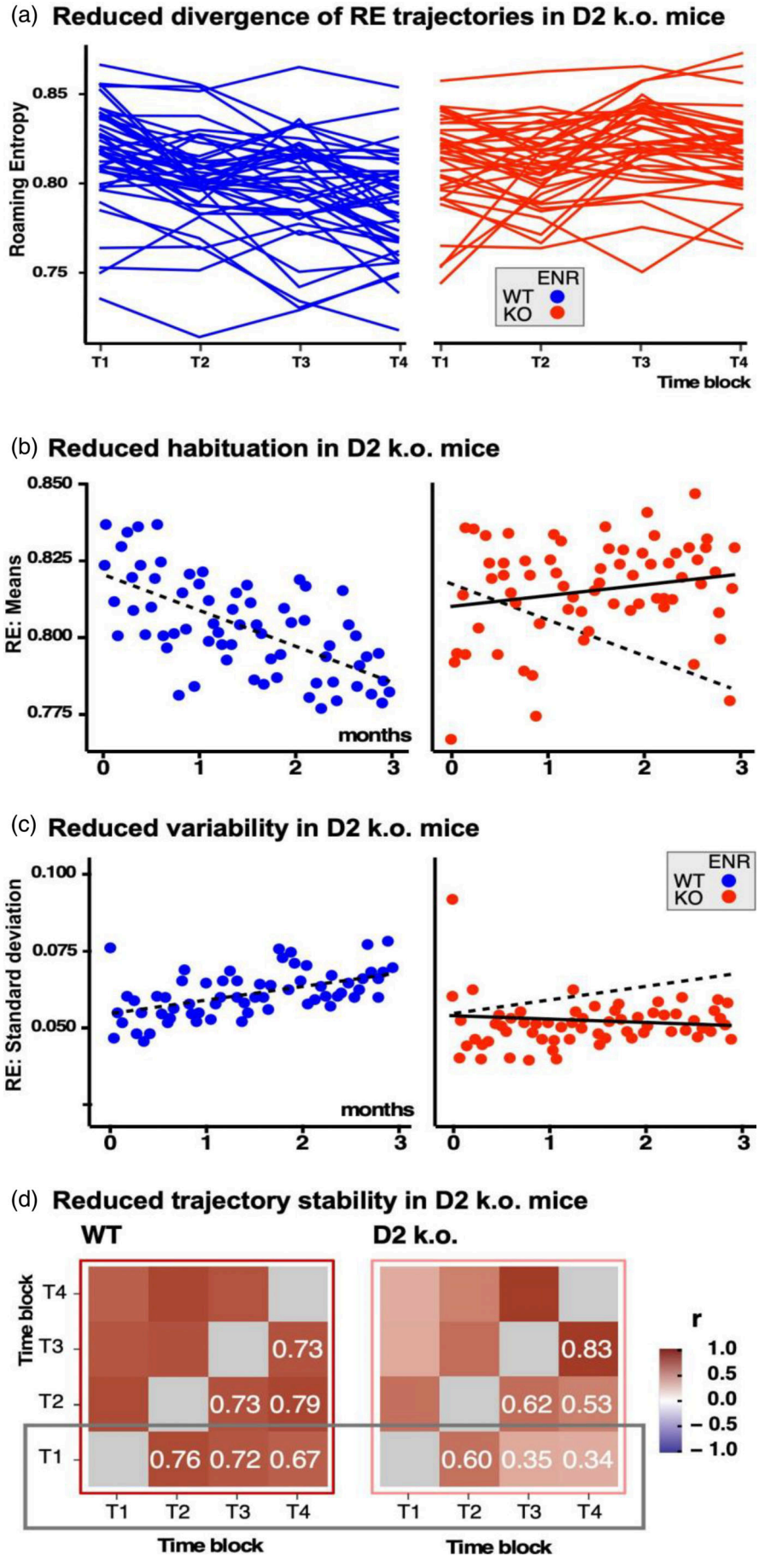

FIGURE 4 Reduced development of individualized behavioral trajectories in D2 knockout mice. (a) Roaming entropy (RE) was calculated from nightly raw antenna counts and binned for better visualization and the calculation of stability (d). Even upon visual inspection, the trajectories of WT versus D2 mice cluster. (b) This is confirmed when plotting daily mean RE levels: habituation was reduced in the knockout animals. (c) At the same time variance of daily RE decreased. The dashed lines always show the WT values. (d) In D2 mice RE in early time blocks was less predictive of RE in later time blocks (Pearson's correlation) than in WT. The two matrices differ statistically significantly (see details in the main text).

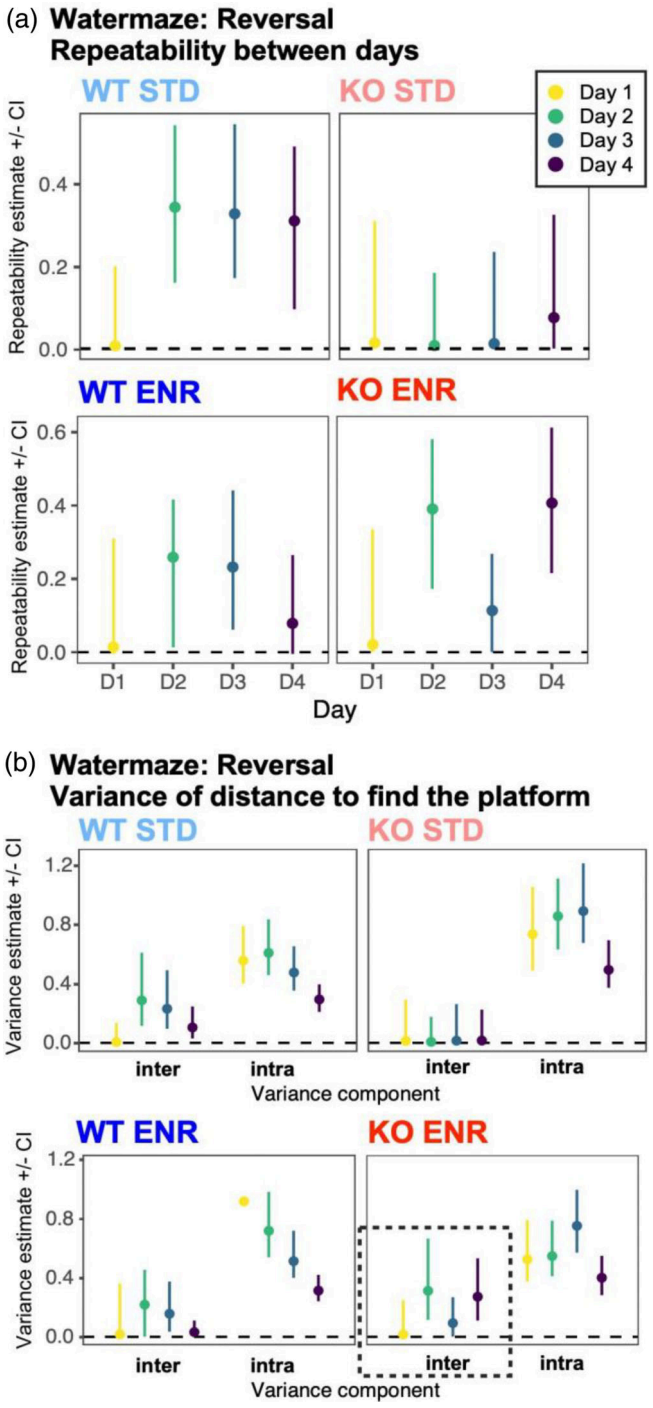

FIGURE 5 Estimates of inter- versus intraindividual variability. The figure shows estimates of repeatability, that is the fraction of the total variance that is attributable to interindividual differences rather than intraindividual variation (Dingemanse & Dochtermann, 2013). As previously shown, the reversal phase is particular sensitive to changes in adult neurogenesis (Garthe et al., 2009, 2016). (a) In D2 k.o. mice the repeatability of path length during the reversal phase was reduced, but rescued by enrichment. (b) Estimates of the intra- and interindividual components reveal an effect of ENR on the interindividual component, which, thus, cannot be explained by adult neurogenesis.

genetic and functional properties. This potential confound is therefore difficult to resolve, especially based on published records. In any case, however, the observed effects would still be neurogenesis-dependent, even though with a potential contribution from olfactory bulb neurogenesis.

Interestingly, the only other stem cell population that is affected by the knockout appear to be pancreatic precursor cells and D2 k.o. mice have smaller islets of Langerhans (Georgia et al., 2010). We carefully monitored glucose levels in our mice and found levels within the physiological range (Han et al., 2008). Glucose levels responded to environmental enrichment (Figure 2b) and did not show obvious inter-individual differences (Körholz et al., 2018). No behavioral or structural consequences of slightly increased glucose levels have been reported, so that it seems unlikely that our results are confounded by this pancreatic phenotype. Memory deficits have been described for much higher levels of blood sugar (and only for rats) (Jurdak & Kanarek, 2009). The learning deficits of D2 k.o. mice are also highly specific to the neurogenesis-dependent functions. D2 k.o. mice had originally been described as entirely normal in the Morris Water Maze and Barnes Maze tasks and it required a particular refinement of the test to expose the specific deficit (Filipkowski & Kaczmarek, 2018; Garthe et al., 2014; Garthe & Kempermann, 2013; Jaholkowski et al., 2009; Urbach et al., 2013). D2 k.o. mice do have effects on the reward system (Jaholkowski et al., 2011), but whether this effect is independent of adult neurogenesis is not known. Adult neurogenesis in fact has a well characterized role in addiction as well as in reward learning (Seib et al., 2021). A potential involvement of reward learning in the observed phenotypes is thus obviously an interesting lead to follow. The complexity of how adult-born neurons contribute to brain function (Kempermann, 2022) suggests anyway that the here-proposed role of new neurons in the development of individualized behavioral trajectories might involve different neurogenesis-dependent (as well as -independent) mechanisms.

Learning and the flexibility in learning are obvious prime candidate mechanisms, but little is known today, to which extent the network changes that new neurons bring to the hippocampus explain variation in hippocampal learning. Fitting to this idea, D2 k.o. mice have been proposed as animal model with high construct validity for schizophrenia (Grimm et al., 2018). That report, surprisingly, did not go into detail regarding adult neurogenesis, but rather focused on an impaired function of parvalbumin-positive interneurons in the hippocampus. It is tempting to speculate, given the role of parvalbumin-positive interneurons and their feedback loops in the control of adult neurogenesis, that this impairment is a consequence of the neurogenesis-knockdown that might help to explain also the phenotype we describe here. Data from our group in collaboration with Hayder Amin, Dresden, indicate that network effects of enrichment are massive and include effects that can be ascribed to the adult-born neurons (preprint under Emery et al., 2022). While those observations require further exploration and confirmation they support the idea that adult-born granule cells have far-reaching consequences for the circuitry and hence functions of the entire brain.

The key phenotype we describe here is that the RE trajectories of D2 k.o. mice do not show habituation and no increase in variance over time. Interindividual correlations were lower in D2 k.o. than in WT at the beginning of the experiment (Figure 4), but increased over time (from $r = 0.6$ to 0.83). For adult neurogenesis, the coefficient of

correlation (r) was lower in the present study than in the our first report (Freund et al., 2013), where it was 0.22, but this might be explainable by the different spatial arrangement of the cages and the antennas. In contrast, the current r^2 was approximately the same as in our other report with the same set-up (Zocher et al., 2020). The difference is thus unlikely to be due to the fact that the two genotypes were mixed in the current study or the WT genotype itself (which is C57BL/6 as in the other study). WT, in contrast, showed more stable behaviors from the start. These findings can be interpreted as indicating that the loss of adult neurogenesis results in a reduced and, in the course, more rigid individual adaptation to the environment. Such interpretation might be consistent with the view that adult neurogenesis favors novelty seeking (Lemaire et al., 1999) and facilitates the flexible integration of novelty into pre-existing contexts (Garthe et al., 2009). The predictability is not lost in the D2 k.o. mice, however, as it is similar at late time blocks 3 and 4 but the pattern is different at early stages (Figure 4d).

In the water maze, the inter-individual component of the variance estimate in the neurogenesis-dependent feature that is found during the reversal phase of the task decreased (Figure 5). The inter-individual component shows how consistently is the behavior of an animal in comparison to its peers, thus only possible to observe in the population context (Dingemanse et al., 2010). Repeatability, a ratio between the inter-individual component of variance and the total variance, indicates the existence of individuality.

D2 k.o. mice under standard housing had zero repeatability estimates during the reversal watermaze phase (Figure 5), indicating that newborn neurons are necessary for behavioral spatial navigation consistency. The observed partial rescue by enrichment (Figure 5) must be explained by enrichment effects that are not mediated by adult-born neurons and, hence, represent an interesting compensation. Notably, such compensation was not observed in the RE trajectories.

Our data do not allow the strong conclusion that individual behavioral trajectories are solely dependent on adult neurogenesis, but they support the working hypothesis that adult-born neurons play a critical role in certain aspects of individualization.

Follow-up studies will have to address our question of how adult neurogenesis is involved in individuality at the level of functional networks and must corroborate our findings with additional strategies to manipulate adult neurogenesis. Several components of environmental enrichment such as physical activity (Kronenberg et al., 2003; van Praag et al., 1999), social interaction (Moreno-Jiménez et al., 2019) and—most likely—learning as such stimulate adult neurogenesis, presumably altering the hippocampal network such that it contributes to adapted behavioral patterns, which in turn sustain a feedback loop (Kempermann, 2002).

But our results indicate, as hypothesized previously (Kempermann, 2019), that newborn granule cells in the adult dentate gyrus play a critical role in processes of brain plasticity that result in inter-individual differences in brain function and the underlying connectome.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw data from the Colony Rack cage system are available at: https://doi.org/10.5061/dryad.5hqbzkh9z

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REFERENCES

Ansorg, A., Witte, O. W., & Urbach, A. (2012). Age-dependent kinetics of dentate gyrus neurogenesis in the absence of cyclin D2. *BMC Neuroscience*, *13*, 46.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Ben Abdallah, N. M.-B., Filipkowski, R. K., Pruschy, M., Jaholkowski, P., Winkler, J., Kaczmarek, L., & Lipp, H.-P. (2013). Impaired long-term memory retention: Common denominator for acutely or genetically reduced hippocampal neurogenesis in adult mice. *Behavioural Brain Research*, *252*, 275–286.

Brown, J., Cooper-Kuhn, C. M., Kempermann, G., Van Praag, H., Winkler, J., Gage, F. H., & Kuhn, H. G. (2003). Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *The European Journal of Neuroscience*, *17*, 2042–2046.

Diamond, M. C. (1988). *Enriching heredity*. The Free Press.

Dingemanse, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: Mixed-effect modelling approaches. *The Journal of Animal Ecology*, *82*, 39–54.

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: Animal personality meets individual plasticity. *Trends in Ecology & Evolution*, *25*, 81–89.

Dochtermann, N. A., & Royauté, R. (2019). The mean matters: Going beyond repeatability to interpret behavioural variation. *Animal Behaviour*, *153*, 147–150.

Emery, B. A., Hu, X., Khanzada, S., Kempermann, G., & Amin, H. (2022). Rich experience boosts functional connectome and high-dimensional coding in hippocampal network. *BioRxiv*. 2022.02.23.480123. https://doi.org/10.1101/2022.02.23.480123

Filipkowski, R. K., & Kaczmarek, L. (2018). Severely impaired adult brain neurogenesis in cyclin D2 knock-out mice produces very limited phenotypic changes. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *80*, 63–67.

Fong, Y., Rue, H., & Wakefield, J. (2010). Bayesian inference for generalized linear mixed models. *Biostatistics*, *11*, 397–412.

Freund, J., Brandmaier, A. M., Lewejohann, L., Kirste, I., Kritzler, M., Krüger, A., Sachser, N., Lindenberger, U., & Kempermann, G. (2013). Emergence of individuality in genetically identical mice. *Science*, *340*, 756–759.

Freund, J., Brandmaier, A. M., Lewejohann, L., Kirste, I., Kritzler, M., Krüger, A., Sachser, N., Lindenberger, U., & Kempermann, G. (2015). Association between exploratory activity and social individuality in genetically identical mice living in the same enriched environment. *Neuroscience*, *309*, 140–152.

Garthe, A., Behr, J., & Kempermann, G. (2009). Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. *PLoS One*, *4*, e5464.

Garthe, A., Huang, Z., Kaczmarek, L., Filipkowski, R. K., & Kempermann, G. (2014). Not all water mazes are created equal: Cyclin D2 knockout mice with constitutively suppressed adult hippocampal neurogenesis do show specific spatial learning deficits. *Genes, Brain, and Behavior*, *13*, 357–364.

Garthe, A., & Kempermann, G. (2013). An old test for new neurons: Refining the Morris water maze to study the functional relevance of adult hippocampal neurogenesis. *Frontiers in Neuroscience*, *7*, 63.

Garthe, A., Roeder, I., & Kempermann, G. (2016). Mice in an enriched environment learn more flexibly because of adult hippocampal neurogenesis. *Hippocampus*, *26*, 261–271.

Georgia, S., Hinault, C., Kawamori, D., Hu, J., Meyer, J., Kanji, M., Bhushan, A., & Kulkarni, R. N. (2010). Cyclin D2 is essential for the compensatory beta-cell hyperplastic response to insulin resistance in rodents. *Diabetes*, *59*, 987–996.

Grimm, C. M., Aksamaz, S., Schulz, S., Teutsch, J., Sicinski, P., Liss, B., & Kätzel, D. (2018). Schizophrenia-related cognitive dysfunction in the cyclin-D2 knockout mouse model of ventral hippocampal hyperactivity. *Translational Psychiatry*, *8*, 212.

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm *R* package. *Journal of Statistical Software*, *33*, 1–22.

Han, B. G., Hao, C.-M., Tchekneva, E. E., Wang, Y.-Y., Lee, C. A., Ebrahim, B., Harris, R. C., Kern, T. S., Wasserman, D. H., Breyer, M. D., & Qi, Z. (2008). Markers of glycemic control in the mouse: Comparisons of 6-h- and overnight-fasted blood glucoses to Hb A1c. *American Journal of Physiology. Endocrinology and Metabolism*, *295*, E981–E986.

Han, W., Umekawa, T., Zhou, K., Zhang, X.-M., Ohshima, M., Dominguez, C. A., Harris, R. A., Zhu, C., & Blomgren, K. (2016). Cranial irradiation induces transient microglia accumulation, followed by long-lasting inflammation and loss of microglia. *Oncotarget*, *7*, 82305–82323.

Jaholkowski, P., Kiryk, A., Jedynak, P., Ben Abdallah, N. M., Knapska, E., Kowalczyk, A., Piechal, A., Blecharz-Klin, K., Figiel, I., Lioudyno, V., Widy-Tyszkiewicz, E., Wilczynski, G. M., Lipp, H.-P., Kaczmarek, L., & Filipkowski, R. K. (2009). New hippocampal neurons are not obligatory for memory formation; cyclin D2 knockout mice with no adult brain neurogenesis show learning. *Learning & Memory*, *16*, 439–451.

Jaholkowski, P., Mierzejewski, P., Zatorski, P., Scinska, A., Sienkiewicz-Jarosz, H., Kaczmarek, L., Samochowiec, J., Filipkowski, R. K., & Bienkowski, P. (2011). Increased ethanol intake and preference in cyclin D2 knockout mice. *Genes, Brain, and Behavior*, *10*, 551–556.

Jurdak, N., & Kanarek, R. B. (2009). Sucrose-induced obesity impairs novel object recognition learning in young rats. *Physiology & Behavior*, *96*, 1–5.

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., & Gage, F. H. (2003). Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development*, *130*, 391–399.

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature*, *386*, 493–495.

Kempermann, G., Lopes, J. B., Zocher, S., Schilling, S., Ehret, F., Garthe, A., Karasinsky, A., Brandmaier, A. M., Lindenberger, U., Winter, Y., & Overall, R. W. (2022). The individuality paradigm: Automated longitudinal activity tracking of large cohorts of genetically identical mice in an enriched environment. *Neurobiology of Disease*, *175*, 105916.

Kempermann, G. (2002). Why new neurons? Possible functions for adult hippocampal neurogenesis. *The Journal of Neuroscience*, *22*, 635–638.

Kempermann, G. (2019). Environmental enrichment, new neurons and the neurobiology of individuality. *Nature Reviews. Neuroscience*, *20*, 235–245.

Kempermann, G. (2022). What is adult hippocampal neurogenesis good for? *Frontiers in Neuroscience*, *16*, 852680.

Körholz, J. C., Zocher, S., Grzyb, A. N., Morisse, B., Poetzsch, A., Ehret, F., Schmied, C., & Kempermann, G. (2018). Selective increases in inter-individual variability in response to environmental enrichment in female mice. *eLife*, *7*, e35690.

Kowalczyk, A., Filipkowski, R. K., Rylski, M., Wilczynski, G. M., Konopacki, F. A., Jaworski, J., Ciemerych, M. A., Sicinski, P., & Kaczmarek, L. (2004). The critical role of cyclin D2 in adult neurogenesis. *The Journal of Cell Biology*, *167*, 209–213.

Kronenberg, G., Reuter, K., Steiner, B., Brandt, M. D., Jessberger, S., Yamaguchi, M., & Kempermann, G. (2003). Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *The Journal of Comparative Neurology*, *467*, 455–463.

Leiter, O., Seidemann, S., Overall, R. W., Ramasz, B., Rund, N., Schallenberg, S., Grinenko, T., Wielockx, B., Kempermann, G., & Walker, T. L. (2019). Exercise-induced activated platelets increase adult hippocampal precursor proliferation and promote neuronal differentiation. *Stem Cell Reports*, *12*, 667–679.

Lemaire, V., Aurousseau, C., Le Moal, M., & Abrous, D. N. (1999). Behavioural trait of reactivity to novelty is related to hippocampal neurogenesis. *The European Journal of Neuroscience*, *11*, 4006–4014.

McQuail, J. A., Dunn, A. R., Stern, Y., Barnes, C. A., Kempermann, G., Rapp, P. R., Kaczorowski, C. C., & Foster, T. C. (2020). Cognitive reserve in model systems for mechanistic discovery: The importance of longitudinal studies. *Frontiers in Aging Neuroscience*, *12*, 607685.

Mohammed, A. H., Zhu, S. W., Darmopil, S., Hjerling-Leffler, J., Ernfors, P., Winblad, B., Diamond, M. C., Eriksson, P. S., & Bogdanovic, N. (2002). Environmental enrichment and the brain. *Progress in Brain Research*, *138*, 109–133.

Monje, M. L., Mizumatsu, S., Fike, J. R., & Palmer, T. D. (2002). Irradiation induces neural precursor-cell dysfunction. *Nature Medicine*, *8*, 955–962.

Moreno-Jiménez, E. P., Jurado-Arjona, J., Ávila, J., & Llorens-Martín, M. (2019). The social component of environmental enrichment is a pro-neurogenic stimulus in adult c57BL6 female mice. *Frontiers in Cell and Development Biology*, *7*, 62.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews. Neuroscience*, *7*, 697–709.

Overall, R. W., Zocher, S., Garthe, A., & Kempermann, G. (2020). Rtrack: A software package for reproducible automated water maze analysis. *BioRxiv*. 2020.02.27.967372. https://doi.org/10.1101/2020.02.27.967372

Perneczky, R., Kempermann, G., Korczyn, A. D., Matthews, F. E., Ikram, M. A., Scarmeas, N., Chetelat, G., Stern, Y., & Ewers, M. (2019). Translational research on reserve against neurodegenerative disease: Consensus report of the international conference on cognitive reserve in the dementias and the Alzheimer's association reserve, resilience and protective factors professional interest area working groups. *BMC Medicine*, *17*, 47.

Petkova, S. P., Pride, M., Klocke, C., Fenton, T. A., White, J., Lein, P. J., Ellegood, J., Lerch, J. P., Silverman, J. L., & Waldau, B. (2020). Cyclin D2-knock-out mice with attenuated dentate gyrus neurogenesis have robust deficits in long-term memory formation. *Scientific Reports*, *10*, 8204.

Płatek, R., Rogujski, P., Mazuryk, J., Wiśniewska, M. B., Kaczmarek, L., & Czupryn, A. (2022). Impaired generation of transit-amplifying progenitors in the adult subventricular zone of cyclin D2 knockout mice. *Cells*, *11*, 135.

Plomin, R., & Daniels, D. (2011). Why are children in the same family so different from one another? *International Journal of Epidemiology*, *40*, 563–582.

van Praag, H., Kempermann, G., & Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience*, *2*, 266–270.

Richter, S. H., Garner, J. P., & Würbel, H. (2009). Environmental standardization: Cure or cause of poor reproducibility in animal experiments? *Nature Methods*, *6*, 257–261.

Seib, D. R., Espinueva, D. F., Princz-Lebel, O., Chahley, E., Stevenson, J., O'Leary, T. P., Floresco, S. B., & Snyder, J. S. (2021). Hippocampal neurogenesis promotes preference for future rewards. *Molecular Psychiatry*, *26*, 6317–6335.

Stern, Y., Arenaza-Urquijo, E. M., Bartrés-Faz, D., Belleville, S., Cantilon, M., Chetelat, G., Ewers, M., Franzmeier, N., Kempermann, G., Kremen, W. S., Okonkwo, O., Scarmeas, N., Soldan, A., Udeh-Momoh, C., Valenzuela, M., Vemuri, P., Vuoksimaa, E., & the Reserve, Resilience and Protective Factors PIA Empirical Definitions and Conceptual Frameworks Workgroup. (2020). Whitepaper: Defining and investigating cognitive reserve, brain reserve, and brain maintenance. *Alzheimers Dementia*, *16*, 1305–1311.

Stern, Y., Chételat, G., Habeck, C., Arenaza-Urquijo, E. M., Vemuri, P., Estanga, A., Bartrés-Faz, D., Cantillon, M., Clouston, S. A. P., Elman, J. A., Gold, B. T., Jones, R., Kempermann, G., Lim, Y. Y., van Loenhoud, A., Martínez-Lage, P., Morbelli, S., Okonkwo, O., Ossenkoppele, R., … Vuoksimaa, E. (2019). Mechanisms underlying resilience in ageing. *Nature Reviews. Neuroscience*, *20*, 246.

Team RC. 2014. *R: A language and environment for statistical computing*. Available from: http://www.R-project.org

Tschen, S.-I., Zeng, C., Field, L., Dhawan, S., Bhushan, A., & Georgia, S. (2017). Cyclin D2 is sufficient to drive β cell self-renewal and regeneration. *Cell Cycle*, *16*, 2183–2191.

Turkheimer, E., & Waldron, M. (2000). Nonshared environment: A theoretical, methodological, and quantitative review. *Psychological Bulletin*, *126*, 78–108.

Urbach, A., Robakiewicz, I., Baum, E., Kaczmarek, L., Witte, O. W., & Filipkowski, R. K. (2013). Cyclin D2 knockout mice with depleted adult neurogenesis learn Barnes maze task. *Behavioral Neuroscience*, *127*, 1–8.

Wickham, H. (2011). ggplot2. *WIREs Comp Stat*, *3*, 180–185.

Wolfer, D. P., Madani, R., Valenti, P., & Lipp, H. P. (2001). Extended analysis of path data from mutant mice using the public domain software Wintrack. *Physiology & Behavior*, *73*, 745–753.

Zocher, S., Schilling, S., Grzyb, A. N., Adusumilli, V. S., Bogado Lopes, J., Günther, S., Overall, R. W., Winter, Y., & Kempermann, G. (2020). Early-life environmental enrichment generates persistent individualized behavior in mice. *Science Advances*, *6*, eabb1478.

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