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# Presymptomatic Reduction of Individuality in the App$^{NL-F}$ Knockin Model of Alzheimer's Disease

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## ABSTRACT

**BACKGROUND:** One-third of the risk for Alzheimer's disease is explained by environment and lifestyle, but Alzheimer's disease pathology might also affect lifestyle and thereby impair the individual potential for health behavior and prevention.

**METHODS:** We examined in mice how the App$^{NL-F/NL-F}$ (NL-F) knockin mutation affects the presymptomatic response to environmental enrichment (ENR) as an experimental paradigm addressing nongenetic factors. We assessed the emergence of interindividual phenotypic variation under the condition that both the genetic background and the shared environment were held constant, thereby isolating the contribution of individual behavior (nonshared environment).

**RESULTS:** After 4 months of ENR, the mean and variability of plasma ApoE were increased in NL-F mice, suggesting a presymptomatic variation in pathogenic processes. Roaming entropy as a measure of behavioral activity was continuously assessed with radiofrequency identification (RFID) technology and revealed reduced habituation and variance in NL-F mice compared with control animals, which do not carry a Beyreuther/Iberian mutation. Intraindividual variation decreased, while behavioral stability was reduced in NL-F mice. Seven months after discontinuation of ENR, we found no difference in plaque size and number, but ENR increased variance in hippocampal plaque counts in NL-F mice. A reactive increase in adult hippocampal neurogenesis in NL-F mice, known from other models, was normalized by ENR.

**CONCLUSIONS:** Our data suggest that while NL-F has early effects on individual behavioral patterns in response to ENR, there are lasting effects on cellular plasticity even after the discontinuation of ENR. Hence, early behavior matters for maintaining individual behavioral trajectories and brain plasticity even under maximally constrained conditions.

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While the genetic risk for developing Alzheimer's disease (AD) has been estimated to be two-thirds, the remaining risk can be attributed to modifiable risk factors (1). Targeting these factors for prevention reduces the danger that patients with AD will overwhelm health care systems worldwide. Particularly, counteracting physical inactivity and low educational attainment has a very high potential for prevention (2–4). However, the reality of beneficial lifestyles is much more complex than what is reflected in popularly recommended interventions (5,6). In addition, the timing of lifestyle intervention is crucial because symptomatic stages might be too late for successful intervention (7–9). Beneficial lifestyles would have to be adopted early.

To understand individual behavioral foundations of resilience, we used a unique experimental paradigm [for a review, see (10)] that exposes the behavioral (nonshared) component of the environmental factor in phenotypic variation (11,12), when genes are held constant (13). With this paradigm, we showed that exploratory activity is linked to brain plasticity (13,14). Individualizing effects on behavior and adult hippocampal neurogenesis were lasting and associated with epigenetic changes in genes involved in plasticity (15). To some extent, adult neurogenesis was necessary for the stable behavioral trajectory to form (16).

Impaired adult neurogenesis has been discussed as a mechanism contributing to memory deficits in AD, although some controversy exists over adult neurogenesis as a compensatory mechanism and its association with plaque pathology and Braak stage (17–20). Adult neurogenesis is an individualizing trait, but it is affected by the transgenic overexpression of AD-related genes, which may obscure behavior-dependent changes. Therefore, we used a second-generation mouse model of AD, in which artificial overexpression is not a confound, as the knockin of human mutations associated with AD into the physiologically regulated mouse amyloid-precursor protein (App) gene (21). Young App knockin mice can be considered as models of preclinical AD and allow for the study of the early influence of environmental factors. The App$^{NL-F/NL-F}$ knockin mice carry the Swedish (NL) and Beyreuther/Iberian (F) mutation in the App gene, leading to elevated levels of amyloid-β (Aβ) 42





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and an increased ratio of A$\beta_{42}$ to A$\beta_{40}$ (20). App$^{NL-F/NL-F}$ knockin mice (henceforth referred to as NL-F mice) slowly start to develop Aβ plaques by age 6 months and the first cognitive impairments by 18 months. App$^{NL/NL}$ mice (henceforth referred to as NL mice) only have the Swedish mutation, which has no impact on the disease phenotypes (21), and therefore can be used as a genetic control for NL-F while not fully representing a wild-type control (22).

To study individual behavioral trajectories, the mice were kept in a large enrichment (ENR) enclosure, which consists of 70 connected standard cages, from ages 6 weeks to 23 weeks (Figure 1B). They were constantly tracked with radiofrequency identification transponders that were injected under their skin in the neck. After 4 months, the mice were tested behaviorally and then kept in standard cages to mimic the increasing inactivity with advancing age that is often seen in the Western lifestyle. The brains were analyzed at 13 months (Figure 1A).

We examined the emergence of differences in the individual behavioral trajectories in NL-F mice because such differences might represent an access to mechanisms that can help explain why preclinical stages of AD might already be associated with behaviors that support disease development or, conversely, prevent it. We also examined whether such behavioral differences might have a measurable effect on Aβ pathology and adult neurogenesis (14).

## METHODS AND MATERIALS

### Animal Husbandry

App$^{NL-F/NL-F}$ mice, which carry a humanized Aβ sequence as well as a Swedish (KM670/671NL) and Beyreuther/Iberian (I716F) mutation on a C57BL/6J background (21), were obtained from the Riken Institute. In App$^{NL/NL}$ mice, the Swedish mutation in the humanized Aβ sequence increases Aβ levels without pathology (22). Because the homozygous breeding of the mutant mice did not produce wild-type littermates and the background strain C57BL/6J is divided into multiple substrains with substantial genetic drift and resulting differences in metabolism, genetics, and immune function (23), we used NL mice as the primary genetic control. C57BL/6JRj mice (henceforth referred to as C57 mice) served as a control line for side effects on metabolism. Because no abnormalities were observed, C57 mice were only partially assessed to reduce complexity and increase power. A total of 162 female animals from the 3 genotypes were maintained using a 12-hour light/dark cycle with food and water ad libitum at the Center for Regenerative Therapies, Dresden, Germany. All animals received the same chow (no. V1534; Sniff), with 9% of energy from fat, 24% from protein, and 67% from carbohydrates. The experiment was licensed by Regierungspräsidium Dresden (DD24-5131/354/51). At age 4 weeks, half of the animals were

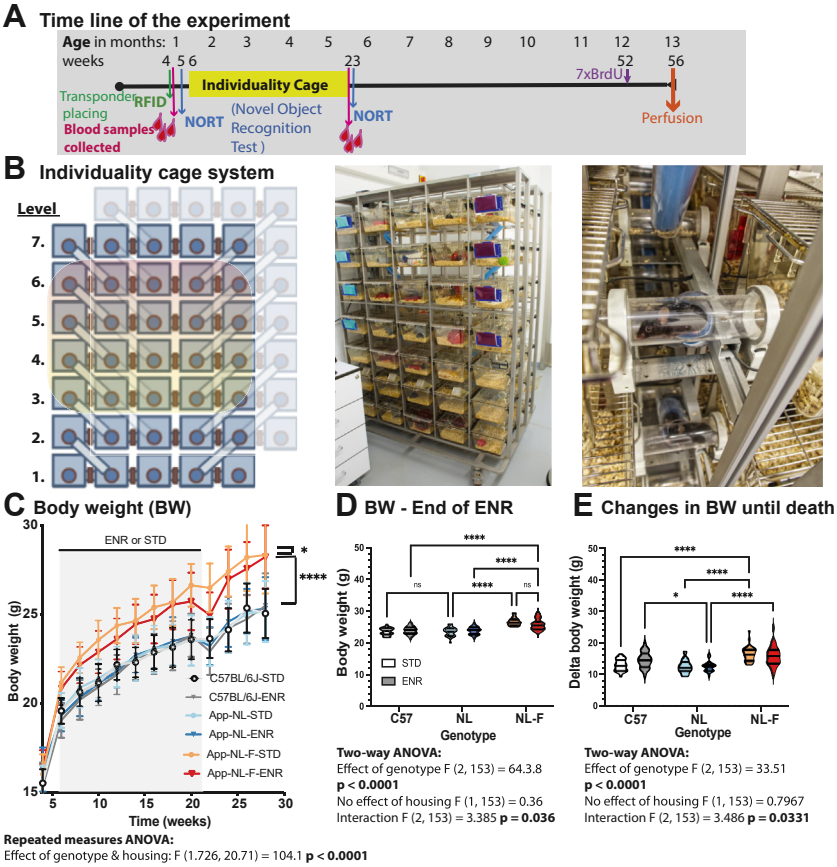


**Figure 1.** Experimental layout and body weight development of mice living in a complex enclosure. **(A)** Experimental timeline outlines the time in the cage system, injections, and analysis parameters. **(B)** Schematic representation (left) and pictures (right) of the individuality cage system for complex ENR and longitudinal monitoring. Each of the 70 cages within the 7 levels contained RFID ring antennas (red) around the tunnels, thereby connecting the different levels and cages next to each other. Space and complexity increased over time from 3 to 7 levels. **(C)** App$^{NL-F}$ mice show elevated body mass already prior to ENR, which reduced upon ENR housing. **(D)** Body weight at the end of ENR at 6 months of age. No effect of variance was determined. **(E)** The gained BW between ages 6 weeks and 13 months did not differ between control mice in either the STD or the ENR condition, whereas the increase in BW in NL-F animals was more pronounced; nevertheless, the variance was not significantly altered. Statistical analysis results are shown below each graph. Interactions are stated as ns, *$p < .05$, ****$p < .005$. ANOVA, analysis of variance; BrdU, bromodeoxyuridine; BW, body weight; ENR, enrichment; ns, not significant; RFID, radiofrequency identification; STD, standard.

randomly assigned to the two housing conditions, either living in ENR enclosure (PhenoSys GmbH, now marketed as PhenoSys ColonyRack Individuality 3.0) (10) or standard housing (STD), with 5 animals per cage. The experimental groups consisted of 27 female animals per condition and genotype, with 81 animals housed together in the same enriched environment and 81 animals in STD separated for each genotype. ENR mice were subcutaneously injected into their neck with a glass-coated microtransponder (SID 102/A/2; Euro ID) under brief isoflurane anesthesia. At that time, blood was collected from the retrobulbar venous plexus of all animals. At 6 weeks, mice entered into the different housing conditions. Initially, ENR mice used only 3 of 7 levels from this cage-rack system (Figure 1A). Every 2 weeks, a new level was made accessible, and toys (houses, balls, nests, swings, tunnels, and bricks) were changed with respect to position and complexity. No running wheels were used as part of the ENR. Food and water locations remained unchanged. After 17 weeks of ENR, blood samples were taken, and all mice were returned to home cages of 4 or 5 animals until they were 13 months old (Figure 1D). Body weight was monitored biweekly, and glucose levels were measured every 4 weeks using a drop of blood from the tail obtained via Accu-Chek test strips (PZN 6114963; Accu Chek Aviva). All mice received 7 intraperitoneal injections of bromodeoxyuridine (BrdU) (50 mg/kg) (Sigma-Aldrich) at age 12 months with an interval of 24 hours and were killed 28 days later with a mixture of ketamine/xylazine and transcardial perfusion with 0.9% saline and 4% paraformaldehyde. Final blood samples were taken before transcardial perfusion. Brains were left in 4% paraformaldehyde overnight at 4 °C and were transferred to 30% sucrose before being sectioned on a freezing microtome at 40 μm thickness.

### Analysis of Radiofrequency Identification Data

Radiofrequency identification antennas in connecting tunnels monitored behavioral activity longitudinally (Figure 1A). PhenoSoft Control (PhenoSys GmbH) saved antenna and mouse identifiers together with the time stamp of the antenna contact. In total, 116 days/nights were recorded, but there were data losses between the 29th and 42nd days, 44th and 49th days, and 77th and 98th days. Noise reduction and roaming entropy (RE) calculation were performed as previously described (1,2). Because mice are nocturnal animals, only the events recorded during the dark phase were used. Frequencies 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 $RE_{i,t} = -\sum_{j=0}^{k}(\rho_{i,j,t} \log \rho_{i,j,t})/\log(k)$, where $k$ is the number of antennas. Dividing the entropy by $\log(k)$ scales the RE to the range between 0 and 1. Data collected after events that might disturb patterns (cage cleaning, behavioral testing, etc.) were excluded. Cumulative RE was calculated by the addition of mean RE from 8 time blocks.

### Statistics

All experiments were carried out with the experimenter blinded to the experimental group. Descriptive statistics for all analyses can be found in Supplement 2. Statistical analyses were performed using Prism 9 (GraphPad) and R (R Core Team, 2014). In R, for normally distributed measures, we used Welsh's *t* test to compare means and *F* tests to test for equality of variance between groups. For repeated measures (longitudinal data), a linear mixed regression analysis was performed using the lmer function from the lme4 package (24), and *p* values were obtained using the likelihood-ratio test of the full model against the model without the analyzed effects. Brown-Forsythe test was used to compare the variances between groups; the *F*-ratio and its *p* value are reported below the graphs if the analysis was significant. Two-way analysis of variance was applied to identify effects of housing and genotype. Data were visualized using the (truncated) violin plot function (with lines at the median and quartiles) in Prism and the ggplot2 package in R (25). Statistical results are plotted below each graph, and additional descriptive statistics of mean, standard deviation, and *N* for each data plot are listed in Supplement 2. For the histological analysis of BrdU and doublecortin (DCX) as well as for behavioral analysis of an open field (OF) task, a novel object recognition task (NORT), and an object location task, a two-way analysis of variance was carried out using Bonferroni post hoc analysis.

Information on behavioral tests (OF and NORT), immunohistochemical analyses, and additional statistical analyses (linear mixed model) can be found in the Supplement.

## RESULTS

### Presymptomatic ENR Positively Influences Metabolism in NL-F Mice

NL mice differ from NL-F mice only in the disease-promoting Beyreuther/Iberian mutation, but they still carry the Swedish mutation that does not have a functional phenotype. To further rule out a potential interaction effect, which, hypothetically, might be part of a systemic individualizing, we used C57 mice as an additional control group at this stage.

There were no differences between NL mice and C57 mice in terms of body weight (Figure 1C–E), blood glucose (Figure S1 in the Supplement), cholesterol, and apolipoprotein E (ApoE) levels throughout the experiment (Figure 2). Triglyceride levels were slightly elevated (~11%) in NL and NL-F mice prior to ENR (Figure 2C, D). The reasons for this small change could not be identified, and the ~60% reduction after ENR should have had a greater impact on metabolism than what was noted. Longitudinal analysis revealed that NL-F mice were already heavier than NL and C57 mice prior to ENR and remained heavier after ENR (Figure 1D). ENR consistently reduced the body weights of NL-F mice (Figure 1C), but no ENR-induced increase in variance was detected (Figure 1E).

In line with these findings, an ENR-dependent reduction in blood glucose was measured only in NL-F mice compared with STD conditions. Glucose levels readjusted after withdrawal from ENR, but a minimal sustained effect remained significantly different (Figure S1 in the Supplement). There was also a correlation between body weight, exploration behavior, and glucose levels in both lines (Figure 2E). In addition, ENR had a positive impact on serum triglycerides (Figure 2D). Interestingly, ENR resulted in higher levels and variance of ApoE in NL-F mice (Figure 2F). Cholesterol levels were not altered by ENR (Figure 2A, B).

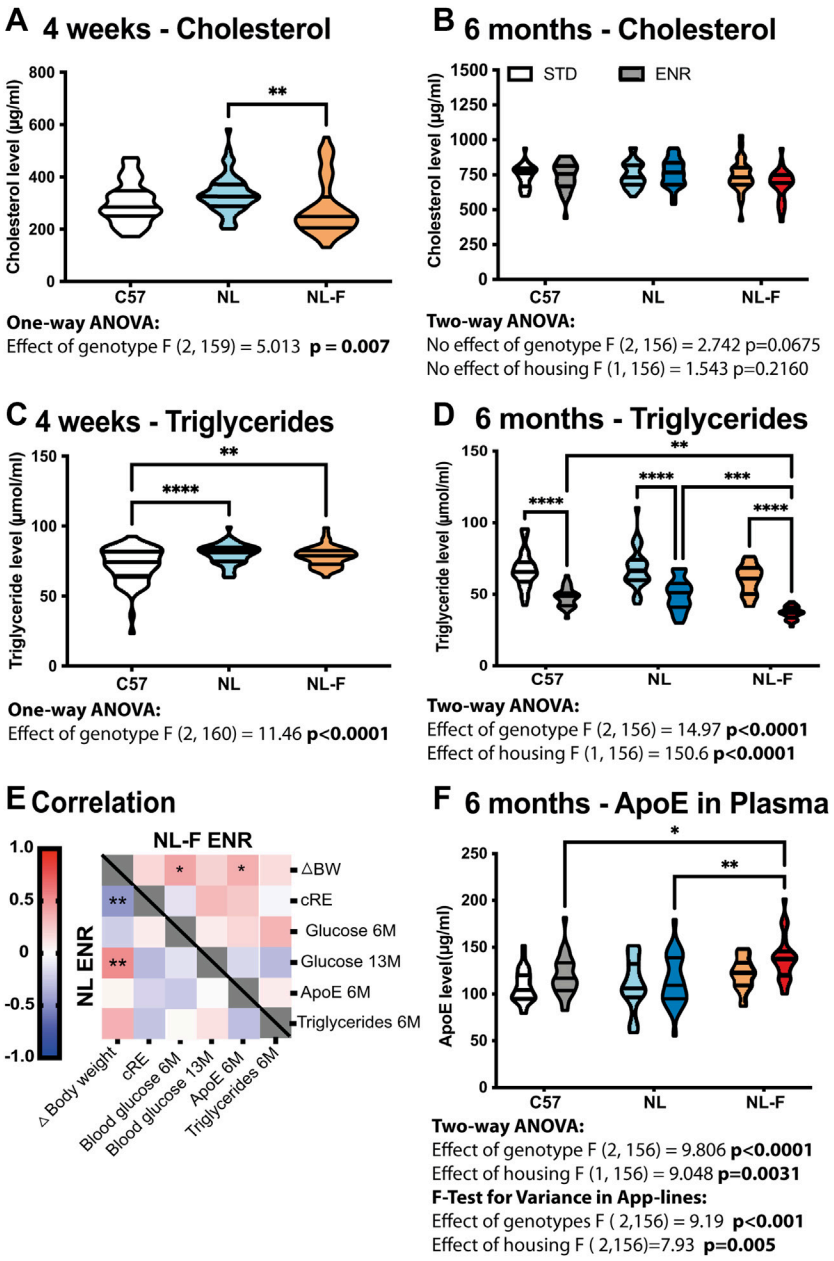


**Figure 2.** Metabolic changes in blood serum before and after complex ENR. **(A, B)** Cholesterol levels did change upon ENR and in NL-F mice. **(C, D)** Triglyceride levels were increased in App lines and reduced upon ENR in all lines. **(E)** Correlation of metabolic parameters and behavioral activity and body weight are shown for both App lines in the ENR cage. A strong correlation between cRE and gain in BW can be seen in the control line. **(F)** ApoE was slightly elevated upon ENR housing in App$^{NL-F/NL-F}$. Significant interactions are shown as $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .005$, $^{****}p < .0001$. ANOVA, analysis of variance; ApoE, apolipoprotein E, BW, body weight; cRE, cumulative roaming entropy; ENR, enrichment; STD, standard housing.

Taken together, these results imply that ENR housing had an impact on metabolism, which is relevant because obesity, increased blood glucose, and elevated triglyceride levels are risk factors for AD (1). ENR affected body weight trajectories and triglyceride levels in NL-F mice, indicating that mice with this genotype are sensitive to ENR stimuli.

In addition, our findings indicate that the Swedish mutation only had minor effects on metabolic parameters (triglycerides and cholesterol increased by 11% prior to ENR but only 1% after ENR compared with C57 mice). Therefore, the NL mice are also a valid control for the NL-F strain for the ENR paradigm, as suggested in the original publications by Saito *et al.* (19). The genetic background of C57BL/6J sublines from different commercial breeders has an impact on immunology, behavior, and metabolism (23,26,27). In contrast, NL and NL-F mice were generated in parallel using the same embryonic stem cell line and similar genetic constructs and originated from the same founder. Furthermore, Salas *et al.* analyzed cognitive function in NL mice until old age and could not detect any cognitive impairment or social deficits; only a small increase in activity was reported (22). Thus, all subsequent analyses were performed using NL mice.

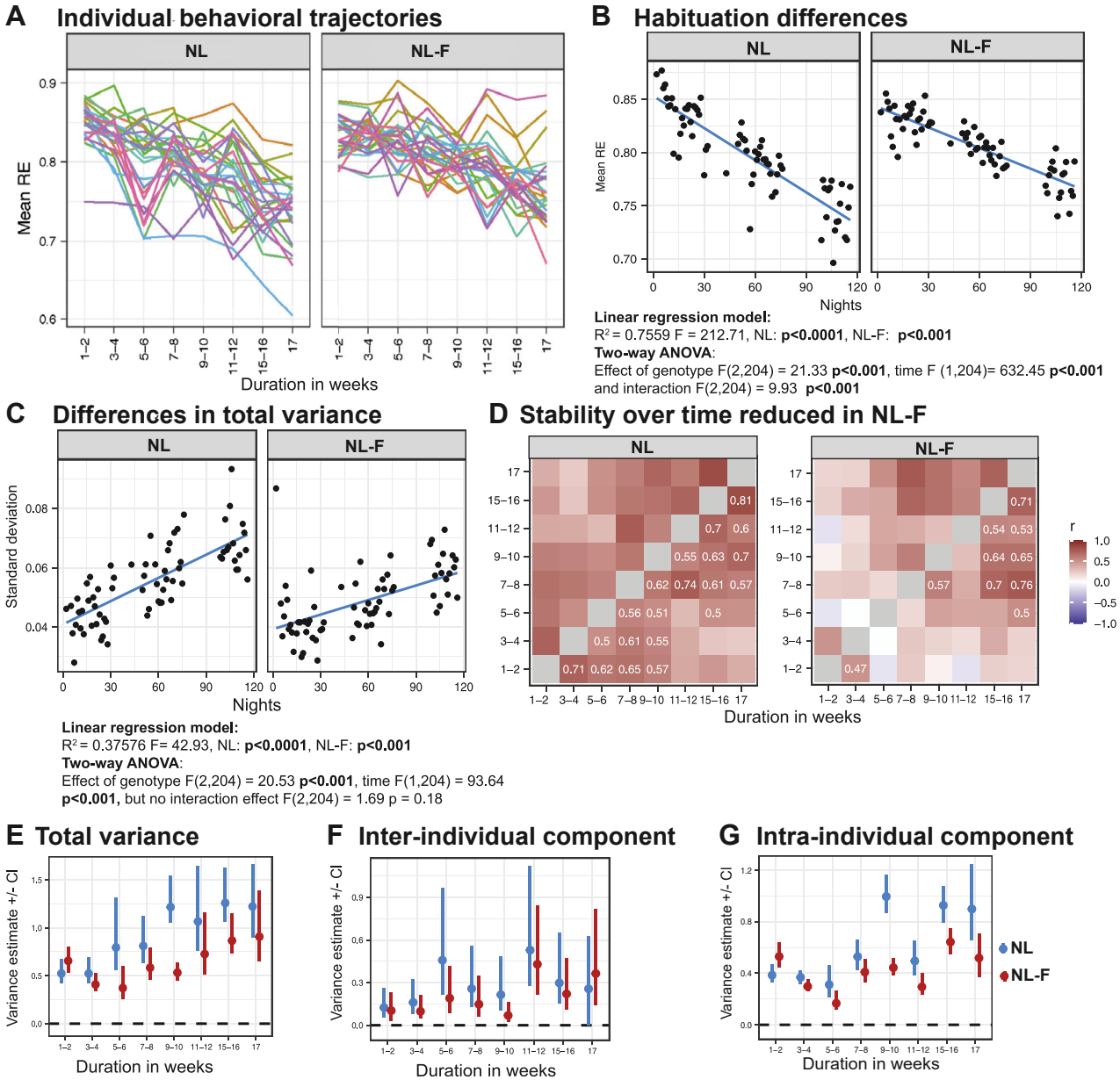


**Figure 3.** Longitudinal analysis of behavioral activity via radiofrequency identification (RFID). **(A)** Individual behavioral trajectories are plotted as RE over time for the different mouse lines. **(B)** Differences in habituation are plotted as changes in mean RE over time in a dot plot. Linear regression analysis on slope of mean RE in NL-F animals. **(C)** NL-F animals have a lower variance than control animals plotted by changes in standard deviation over time. **(D)** Interindividual posterior correlations of RE were significant for larger time frames in NL but not NL-F mice. Thus, the development of stable behavioral trajectories was diminished in NL-F animals as depicted by the correlation matrix, indicating that they are less predictable in their interindividual component. Color code highlights the coefficient of Pearson's *R*. **(E–G)** Detailed analysis of the variance component by a generalized linear mixed model indicates that variance between the animals from the different lines only differs in their intraindividual component **(G)** and not in their interindividual component **(F)**; thus, Alzheimer's disease animals also develop increasing interindividual differences over time. Shown are modes of posterior density with 95% CIs. For the calculation on the generalized linear mixed model, see Table S1 in the Supplement. ANOVA, analysis of variance; RE, roaming entropy.

## NL-F Mice in a Long-term Enriched Environment Show Reduced Stability and Intraindividual Differences in Behavioral Trajectories

To our knowledge, our study has been the first attempt to precisely characterize behavioral changes at an individual level at a presymptomatic stage. Behavioral trajectories were calculated on the basis of mean RE, as the average RE within a time block of 2 weeks. During ENR, NL and NL-F mice developed stable behavioral trajectories with individual differences (Figure 3A) (C57 values are shown side by side in Figure S2 in the Supplement). While the majority of NL animals reduced RE over time as a sign of habituation, the behavioral

trajectories of NL-F animals showed a flatter pattern with less variation (Figure 3A, B). The total variance in RE, represented as the standard deviation, decreased in both strains over time but was less pronounced in NL-F mice (Figure 3C). How stable and predictable mice from the different genotypes become was analyzed by assessing the interindividual correlations of REs between time blocks. Mice usually tend to become more predictable over time, and the coefficient of Pearsons's *R* is greater between later time blocks (15). However, lower correlation coefficients in NL-F mice throughout the experiment indicated a reduced behavioral stability than in control animals (Figure 3D) and reduced adaptation. To evaluate which variance component (variance within an animal or between animals) was changed in NL-F mice, a generalized linear mixed model was applied. For a full description from the Markov chain Monto Carlo analysis on alternative simplified models, see Table S1 in the Supplement (the used code can be also found in Supplement-R-Code-GLMM-Model.R).

While total variance estimates differed over time and NL mice showed a constant increase until 11 weeks, NL-F mice took longer and plateaued only in the last time block (Figure 3E). This effect seemed to be due less to interindividual differences (Figure 3F) than to intraindividual differences (Figure 3G). The intraindividual variance over time was unchanged in NL-F mice and did not increase as in control mice (Figure 3G), further indicating that NL-F individuals may be less flexible.

In conclusion, NL-F mice at this premanifestation stage already showed prominent behavioral differences in longitudinal behavioral patterns. While both genotypes foster a

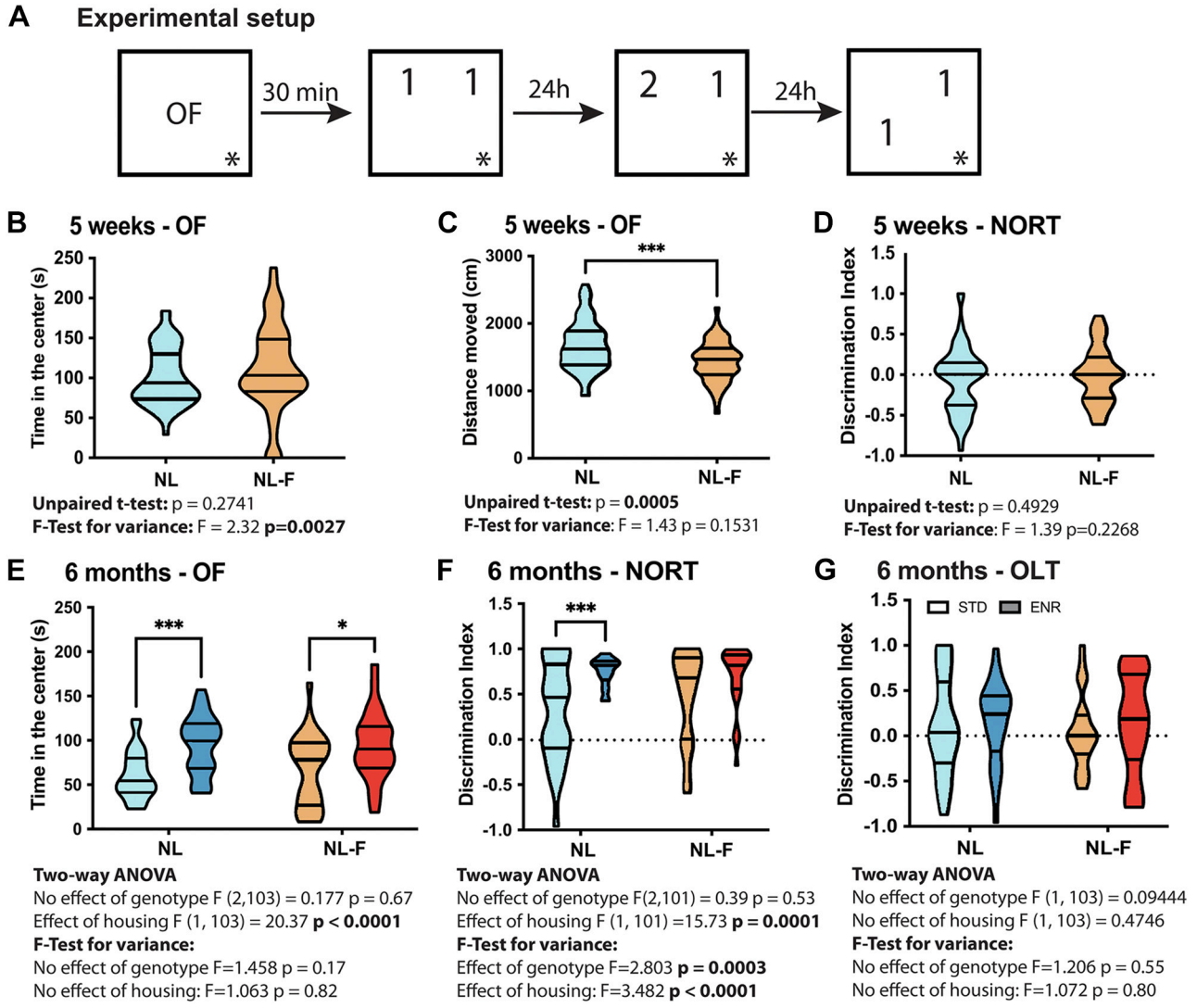


**Figure 4.** ENR increases object recognition and reduces anxiety. **(A)** Experimental setting and object placement for NORT and OLT. **(C)** OF activity is reduced in NL-F mice due to increased body weight. **(B, E)** After ENR, center time is increased as a measure of reduced anxiety. **(D, F)** ENR increases object recognition during NORT in NL but not NL-F mice. **(G)** Object displacement seems to be not improved upon ENR. Significant interactions are shown as $*p < .05$, $***p < .005$. ANOVA, analysis of variance; ENR, enrichment; NORT, novel object recognition task; OF, open field; OLT, object location task; STD, standard housing.

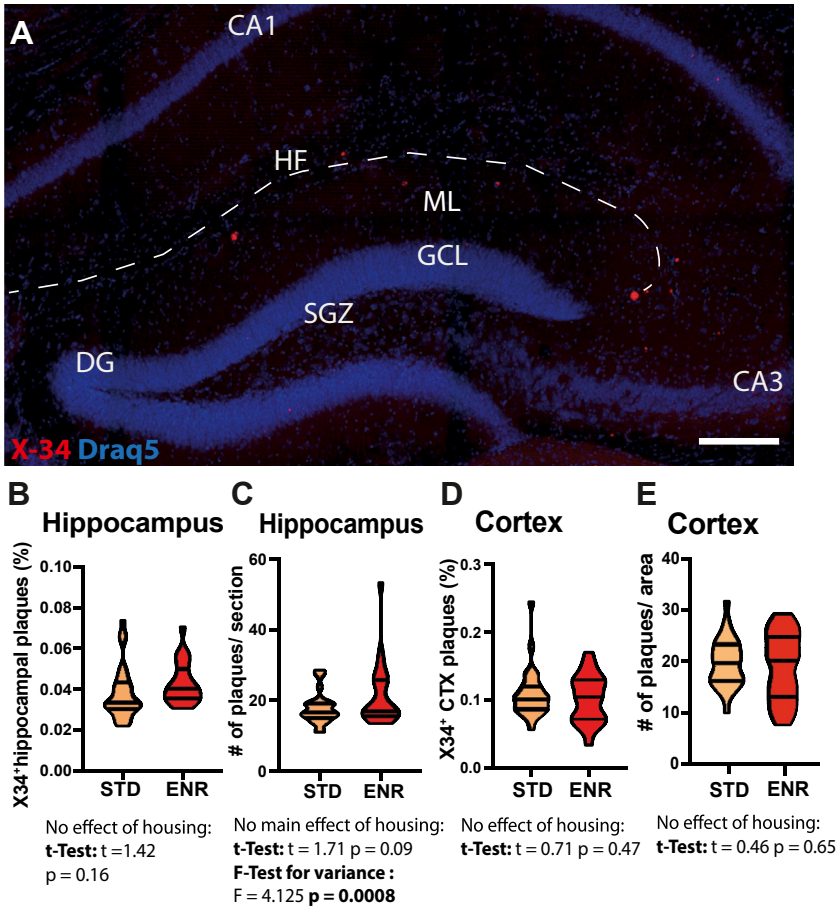


**Figure 5.** Alzheimer's disease–like plaque load at an early presymptomatic stage of 13 months. Plaque load was analyzed by quantification of X-34, a derivate of Congo Red. **(A)** Representative micrograph of plaque load in the hippocampus of NL-F mice, in which plaques are shown in red. Scale bar = 150 μm. **(B, C)** Plaque coverage in the hippocampus is not altered at this early stage in NL-F mice **(B)**, but an increased variance was detected by analyzing the number of plaques in the hippocampus after ENR **(C)**. **(D, E)** A higher plaque load can be seen in the cortex; however, no influence by ENR was detected. DG, dentate gyrus; CTX, cortex; ENR, enrichment; GCL, granule cell layer; HF, hippocampal fissure; ML, molecular layer; SGZ, subgranular zone; STD, standard housing.

comparable capacity to develop a range of individual trajectories, NL-F mice lacked the physiological habituation and individually had become less predictable, exhibited less short-term flexibility, and maintained the same behavioral patterns. The effect on intraindividual stability (or change and fluctuation) creates an individuality that is not captured when focusing on stable residual interindividual differences.

## No Enhanced Object Recognition in Enriched NL-F Mice

Next, we looked for other subtle behavioral changes in NL-F mice at this early stage. The OF task and especially the NORT (Figure 4A) had revealed individualization in previous studies (13,14). Prior to ENR, there were no behavioral differences (Figure 4B, D), except that the activity of NL-F mice was slightly reduced (Figure 4C). After ENR, the amount of time spent in the center of the arena had increased independent of genotype (Figure 4E). NL mice showed an improvement in objection recognition (Figure 4F), but recognition of the displaced object in the object location task was not changed (Figure 4G). In conclusion, the NL-F mutation prevented a positive influence of ENR, even though no impairment was noticeable with STD housing.

NL-F mice started out with an increased variance in the OF prior to ENR (Figure 4B), which was lost at the end of the ENR period (Figure 4E). ENR led to a strong reduction in the variance of the discrimination index in NL mice (Figure 4F), which was not seen in NL-F mice. These findings are not straightforward to interpret in the context of our previous results (1,4), but in NL-F mice, there might be subtle, very early changes in the variance of exploratory behaviors and not only in their mean.

## Increased Presymptomatic Variance in Plaque Numbers

Whether ENR has an impact on amyloid plaque load and size is still under debate and depends on the model being investigated and the timing of the intervention (28,29). Plaque load (Figure 5A) was still very low at the presymptomatic stage of 13 months in NL-F mice, and no ENR effect on plaque burden was detected in the hippocampus (Figure 5B) or cortex (Figure 5D). Nevertheless, there was an ENR-induced increase in the variance in plaque number in the hippocampus (Figure 5C), which supports an individualization of the developing AD pathology.

## Increase in Adult Hippopcampal Neurogenesis

Even though AD in human and AD-like mouse models is associated with a decline of adult neurogenesis at late stages (18,28,30,31), little is known about the preclinical stage because most animal models progress quickly, and human data are

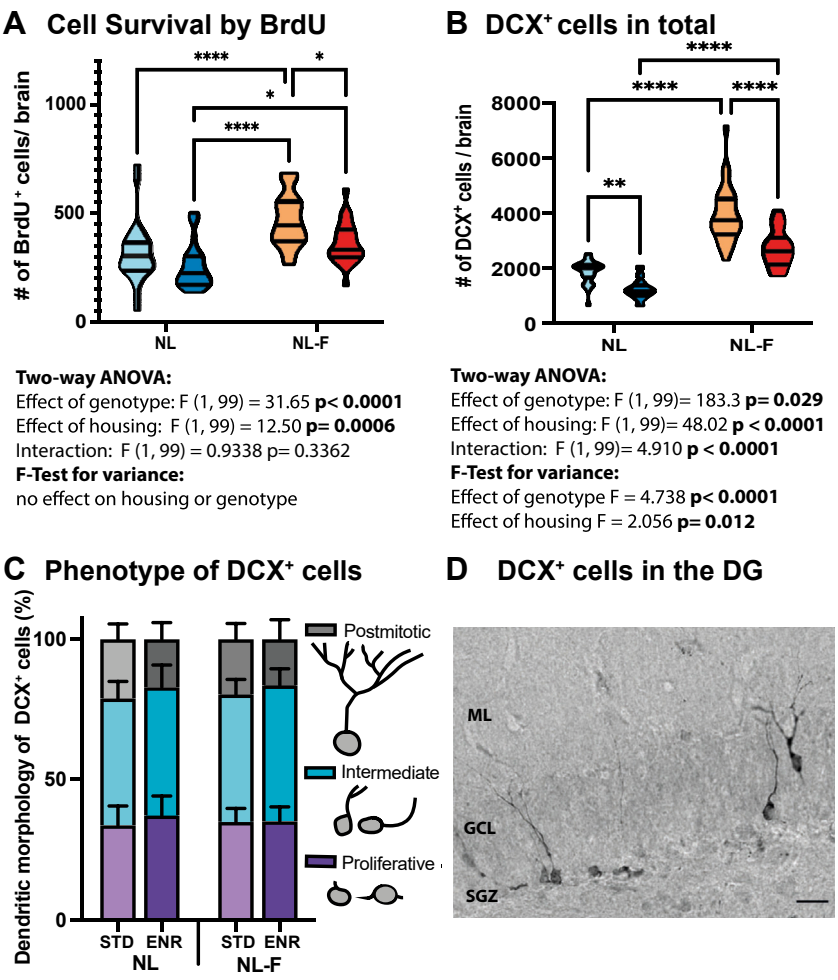


**Figure 6.** Adult neurogenesis in the hippocampus is increased at a presymptomatic stage in App$^{NL-F}$ mice but reduced by environmental ENR. **(A)** The rate of adult neurogenesis in the hippocampus was assessed by BrdU incorporation in dividing cells 28 days prior to perfusion. Adult neurogenesis was increased in NL-F mice at this early stage and reduced in animals withdrawn from ENR. **(B)** The total number of DCX$^+$ cells was increased in NL-F mice and reduced in the ENR group, similar to the BrdU quantification. **(C)** DCX$^+$ cells can be further classified into proliferative, intermediate, and postmitotic phenotype; neither ENR nor the genotype had an effect on the percentage of phenotypic stage. **(D)** Representative micrograph of the DCX$^+$ cells in the DG. Scale bar = 25 μm. **(E)** The number of proliferative DCX$^+$ cells after ENR withdrawal was not altered in control mice but only in NL-F mice. **(F)** The total number of postmitotic DCX$^+$ neurons was influenced by housing and genotype, leading to more cells in NL-F mice and a compensation by ENR. Significant interactions are shown as $^*p < .05$, $^{**}p < .01$, $^{****}p < .0001$. ANOVA, analysis of variance; BrdU, bromodeoxyuridine; DCX$^+$, double-cortin positive; DG, dentate gyrus; ENR, enrichment; GCL, granule cell layer; ML, molecular layer; SGZ, subgranular zone; STD, standard housing.

scarce. The NL-F model revealed an increased number of BrdU$^+$ cells in the granular layer of the dentate gyrus 28 days after the BrdU injections in comparison with the NL model (Figure 6A). To further confirm this observation with an endogenous marker, we evaluated the number and morphology of DCX$^+$ cells (Figure 6D). In NL-F mice, the total number of DCX$^+$ cells had increased (Figure 6B), with no significant alteration in the percentage of proliferative, intermediate, or postmitotic cells (Figure 6C). Thus, the proliferative pool had increased to the same extent as the number of postmitotic cells (Figure 6E, F). This is consistent with the previous observation that the dynamics of maturation in adult neurogenesis is relatively stereotypic (32). The temporary increase in adult neurogenesis may reflect a compensatory response, which is also seen in humans (18), other mouse models (33,34), and zebrafish (35,36).

Exposure to ENR reduced adult neurogenesis at age 12 months, which was 6 months after withdrawal from ENR. Neurogenesis was reduced to even slightly below STD levels. Consequently, the well-described positive neurogenic effects of ENR during adolescence and early adulthood were not sustained into older ages if the stimulation was not reinforced. Nevertheless, other positive effects of ENR such as those on DNA methylation are likely to persist even after withdrawal, as reported previously (24).

## DISCUSSION

To our knowledge, this study was the first of its kind to analyze individual differences in a murine AD model.

We established that complex experiences in early life led to reduced intraindividual differences in behavior. Behavioral patterns in NL-F mice varied less over time than in NL mice. The previously reported effects on an interindividual component of variance was not impaired in NL-F mice. However, NL-F mice became both less predictable and less flexible and showed compromised improvement in object recognition upon ENR even at an otherwise presymptomatic stage. This suggests that AD pathology can weaken early physiological individualization and increase behavioral rigidity. In contrast, the earliest signs of plaque pathology showed greater variance in the hippocampus in NL-F mice under ENR conditions. Perhaps counterintuitively, after withdrawal from ENR, the pathologically enhanced adult neurogenesis that has been reported for several AD models was reduced to slightly below STD levels but remained higher than in NL mice. A direct correlation between individual activity and adult neurogenesis could not be established due to the long withdrawal from ENR.

In an ongoing study comparing ENR effects in young versus old animals, we detected more stable behavioral patterns and reduced intraindividual variance in the older animals (unpublished observations), suggesting that reduced adaptability or behavioral malleability is part of a reduced flexibility at older age. More brain plasticity means greater flexibility and better adaptability, but it might also shift the stability-plasticity balance out of equilibrium (37,38). Thus, young NL-F mice may already show behavioral alterations that are normally seen in older animals. No causal link between RE trajectories and the OF task or NORT can be established at this point, but a more complex task such as the Morris water maze would be more conclusive (but could not be realized for this large cohort).

Incidentally, the feeling of "getting lost" and becoming disoriented to place and time are characteristic of patients with AD even at early stages of the disease (39). Hence, the observed behavioral phenotype might correspond to a very first sign of AD manifestation long before overt plaque pathology and cognitive impairment.

Adult hippocampal neurogenesis might be important for brain maintenance because of the strategic position of the hippocampus in complex learning and memory. We have hypothesized the existence of a neurogenic reserve to cope with aging and neurodegeneration (14,40).

The increase in adult neurogenesis that has been observed in some studies on human AD tissue (17,18) and in murine APP$_{Sw,Ind}$ transgenic overexpression models (33,34) has been interpreted as an initial compensatory attempt. Oligomeric Aβ may directly induce adult neurogenesis (34). Indeed, we found a correlation between plaque load and BrdU levels under both housing conditions (Figure S4 in the Supplement).

It is not clear, however, whether the increase in adult neurogenesis corresponds to an abortive form of regeneration as seen in zebrafish (35). In our study, proliferating DCX$^+$ cells were affected similarly to postmitotic cells so that the increase in proliferation appeared to be associated with an increase in maturing postmitotic neurons (41). We did not observe any ectopic immature neurons (30) as has been discussed for some AD models and well described for the increased proliferation in models of epilepsy (42,43). There was a trend toward a lower number of postmitotic new neurons at 18 months (Figure S3 in the Supplement).

To identify whether changes in metabolism as relevant risk factors manifest already at a preclinical stage in our AD model (44,45), we investigated overall body weight, blood glucose, cholesterol, and triglycerides. The observed increase in body weight in AD animals is in line with the observations in a previous report (45). Higher blood glucose levels in NL-F mice reflected the alterations in glucose metabolism seen in patients with AD (46,47). Because brain glucose hypometabolism precedes the development of cognitive decline in patients with AD (48), an association with higher plasma glucose levels seems likely (49). We found an AD effect on glucose metabolism already during presymptomatic stages.

ENR significantly counteracted the increased body weight in NL-F mice, and the NL-F mice retained a significantly lower mean blood glucose level after withdrawal from ENR. Increased triglyceride levels can be found in conditions like obesity, stroke, and cardiovascular disease (50). Because these are all associated risk factors for AD, the observed decrease in triglycerides in both genotypes will be beneficial (51). In addition, ApoE plays a pivotal role in lipoprotein metabolism and has a strong impact on AD development (52). Nevertheless, some controversy exists with respect to ApoE levels in plasma and the risk for dementia or cardiovascular diseases (53,54). In our study, ENR increased ApoE levels in plasma in comparison with STD, which was most pronounced in NL-F mice. Because triglyceride and cholesterol levels and ApoE are interconnected (55), ENR most likely has a major impact on lipid metabolism, particularly in AD, due to the known interactions between lipid metabolism and AD pathogenic mechanisms. Astonishingly, we found a negative correlation between ApoE levels and plaque area in the hippocampus of mice in ENR but not STD (Figure S4 in the Supplement).

Hence, individual differences in activity and body weight may have a positive impact on metabolism (like blood glucose) and also influence ApoE metabolism, with potential impact on Aβ deposition and plaque burden. Thus, ENR may be able to alleviate symptoms related to metabolism in preclinical AD and thus potentially reduce the impact of related risk factors.

From the small differences in variance between trajectories in ENR, especially during expansion of available cage configuration (weeks 3–10 in Figure 3F), we assume that there are also genotype effects on the interindividual component. This seems plausible from the trajectories in Figure 3A, but our study was underpowered to confirm such differences.

In conclusion, ENR is effective in NL-F mice but did not induce physiologically stable behavioral trajectories long before disease manifestation. The observation that the reduced individuality was mostly explainable by the intra-individual component of variance points to an interesting aspect that had received little attention to date. Individuality also lies in short-term behavioral flexibility along a more or less stable trajectory, not only in differences between those trajectories.

While NL-F mice responded to the behavior-inducing environmental intervention, i.e., the shared environment, the non-shared component showed a reduced effect. This could mean that AD pathology, even at preclinical stages, counteracts behavior that would support the successful buildup of resilience. While the knockin model does not allow straightforward generalization to the human situation, there is a chance of a self-reinforcing pathogenic mechanism in AD that determines the success of a preventive lifestyle by interfering with health behaviors.

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