The Impacts of Early-life Adversity on Striatal and Hippocampal Memory Functions

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Abstract—The impacts of early-life adversity (ELA) on cognitive functions including striatal-dependent habit memory and hippocampal-dependent spatial memory were investigated in male mice. The ELA mouse model was generated via an altered cage environment with limited nesting and bedding materials during postnatal days 2–9 (P2–9). The altered cage environment affected the nesting behaviors of dams, creating a stressful condition for their offspring. The ELA mice had biased decision making and poor spatial memory when they grew into young adults (4-month-old). To explore the underlying synaptic basis of these effects, excitatory synapses represented by postsynaptic density protein-95 (PSD-95) were immunolabelled on a series of brain sections and stereologically quantified in the dorsomedial striatum (DMS) and dorsolateral striatum (DLS), as well as in area CA1 of the dorsal hippocampus. Increased PSD-95-immunoreactive synapses were observed in DLS but not DMS, whereas selective loss of PSD-95 synapses was detected in the stratum radiatum of area CA1. The spine data supported the selective effects of ELA on PSD-95 synapses. Specifically, both thin and mushroom-type spines were increased in DLS, while loss of thin spines was apparent in CA1 radiatum in ELA mice versus controls. The correlation between PSD-95 synapses and memory performances was further analyzed, and the data suggested that increased small (<0.20 μm³) and large (>0.40 μm³) synapses in DLS might drive ELA mice to make decisions largely relying on habit memory, while loss of small synapses in hippocampal CA1 damage the spatial memory of ELA mice.

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INTRODUCTION

Exposure to early-life adversity (ELA) can lead to enduring cognitive deficits and motivational disorders later in life. Clinical studies have consistently identified a higher rate of psychiatric disorders among adult individuals with childhood adversities (Teicher et al., 2016; Birn et al., 2017). Indeed, adverse experiences during early development can sculpt the brain and produce long-term impacts on neural structure and function in both humans and rodents (Brunson et al., 2005; Cirulli et al., 2009; Mueller et al., 2010; Pechtel and Pizzagalli, 2011; Bock et al., 2014; Bick and Nelson, 2016; Chen and Baram, 2016; Masson et al., 2016; Bolton et al., 2019; Xu et al., 2020; Lesuis et al., 2021). Studies from rodents have shown that ELA can cause fundamental changes in several brain regions, including the dorsal hippocampus and the dorsal striatum. Although a distinction has been described between the functioning of the dorsal hippocampus and the dorsal striatum (Packard and McGaugh, 1996; Doeller et al., 2008; Marchette et al., 2011), data from recent studies have indicated that these two distinct structures may dynamically interact to translate hippocampal-associated memory into striatal-associated actions (Ferbinteanu, 2016; Gasser et al., 2020; van de Ven et al., 2020). While the detrimental effects of ELA on cognitive functions including striatal-dependent habit memory and hippocampal-dependent spatial memory were investigated in male mice. The ELA mouse model was generated via an altered cage environment with limited nesting and bedding materials during postnatal days 2–9 (P2–9). The altered cage environment affected the nesting behaviors of dams, creating a stressful condition for their offspring. The ELA mice had biased decision making and poor spatial memory when they grew into young adults (4-month-old). To explore the underlying synaptic basis of these effects, excitatory synapses represented by postsynaptic density protein-95 (PSD-95) were immunolabelled on a series of brain sections and stereologically quantified in the dorsomedial striatum (DMS) and dorsolateral striatum (DLS), as well as in area CA1 of the dorsal hippocampus. Increased PSD-95-immunoreactive synapses were observed in DLS but not DMS, whereas selective loss of PSD-95 synapses was detected in the stratum radiatum of area CA1. The spine data supported the selective effects of ELA on PSD-95 synapses. Specifically, both thin and mushroom-type spines were increased in DLS, while loss of thin spines was apparent in CA1 radiatum in ELA mice versus controls. The correlation between PSD-95 synapses and memory performances was further analyzed, and the data suggested that increased small (<0.20 μm³) and large (>0.40 μm³) synapses in DLS might drive ELA mice to make decisions largely relying on habit memory, while loss of small synapses in hippocampal CA1 damage the spatial memory of ELA mice.
effects of ELA on hippocampal-dependent memory function in adulthood have been extensively reported (e.g., Brunson et al., 2005; Bath et al., 2017; Youssef et al., 2019), the potential impacts of ELA on striatal-dependent habit memory and goal-directed actions remain much less investigated.

The dorsal striatum is a heterogeneous brain structure that can be broadly divided into dorsomedial and dorsolateral subregions (DMS and DLS, respectively). It is generally agreed that DLS is primarily implicated in habitual behavior, whereas DMS is critical to the acquisition and execution of goal-directed actions, mediating the stimulus–response habit learning process (Yin and Knowlton, 2006; Shan et al., 2014; Goodman and Packard, 2018). Recent studies in both humans and rodents have emphasized that the dorsal striatum may be dynamically involved in context-dependent spatial decision making and responding via connection with the hippocampus and frontostriatal loops (Ferbinteanu, 2016; Gasser et al., 2020; van de Ven et al., 2020). It has been shown that medium spiny neurons, the principal cells in the dorsal striatum, receive glutamatergic inputs from the hippocampus in addition to the well-defined dopaminergic inputs (Yager et al., 2015; Hintiryan et al., 2016). Particularly, the glutamatergic afferents make synaptic contacts primarily with the dendritic spines of spiny striatal neurons (Waldvogel et al., 1997; Hintiryan et al., 2016). Therefore, alterations in the hippocampus should impact the excitatory innervation of the dorsal striatum, modifying the synaptic contacts on the striatal neurons. Extensive studies have reported the selective damage and loss of hippocampal neurons provoked by stress early in postnatal life (e.g., Brunson et al., 2005; Bath et al., 2017; Youssef et al., 2019), which may impinge on the post-synaptic target cells in the dorsal striatum, leading to aberrant striatal behavioral outcomes. In line with this notion, studies on rodents have revealed that chronic stress biases decision-making strategies, which is highly associated with the reorganization of neural circuits and cells in the dorsal striatum (Dias-Ferreira et al., 2009; Friedman et al., 2017). Collectively, these reports imply that ELA may impede neural development and synaptic connections across the dorsal striatum and hippocampus with altered consequences for memory and executive functions.

We have reported that ELA via an altered cage environment can delay the maturation of dendritic spines on striatal neurons (He et al., 2021). However, it is unknown whether ELA impacts striatal-dependent functional outcome. Here, we used a limited nesting and bedding paradigm to generate the ELA mouse model and explored the influences of ELA on striatal and hippocampal associated memory functions. Considering that the stress response is sex-dependent, male mice were employed in the current study. We found that an altered cage environment during postnatal days 2–9 (P2–9) affects the nesting behaviors of dams, creating a stressful condition to the pups. Importantly, this type of ELA disrupts decision making and damages spatial memory. Given the relevance of excitatory synaptic connections in cognitive function, synapses represented by postsynaptic density protein-95 (PSD-95) (e.g., Chen et al., 2013; Maras et al., 2014) and dendritic spines were stereologically quantified in the dorsal striatum (DLS and DMS) and area CA1 of the dorsal hippocampus. The correlation between synapses of different sizes and memory performances was further analyzed. The data indicated that increased small and large synapses in DLS might drive mice that experienced ELA during P2–9 to make decisions relying on habit memory, whereas loss of small synapses in hippocampal CA1 damaged the spatial memory of ELA mice.

**EXPERIMENTAL PROCEDURES**

**Animals**

Male C57BL/6J mice were used in the experiments. The parent mice were purchased from Beijing Laboratory Animal Research Center (SCXXK (Jing) 2016-0006). The mice were housed in cages (30 × 15 × 12 cm) with corncob bedding (~400 ml) and two pieces of pressed cotton square (5 × 5 × 0.5 cm). All cages were maintained in a well-ventilated and temperature-controlled room on a 12:12 light/dark cycle (lights on at 7:00 am). The mice had free access to water and standard rodent chow. Parturition was checked daily, and the day of birth was considered postnatal day 0 (P0). On P2, pups from several litters were gathered and 6 pups (3 males and 3 females) were assigned at random to each dam to prevent the potential confounding effects of genetic variables and litter size. Pups were weaned and segregated according to sex at postnatal day 21 (5 mice per cage). Efforts were made to minimize the number of animals used and their suffering. All procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Yangtze University.

**Model of early life adversity (ELA)**

The ELA animal model was generated as described previously with minor modifications (Rice et al., 2008; He et al., 2021). On days 11–13 of gestation, pregnant mice were randomly assigned to ELA and control groups and housed in separate cages. On P2, the ELA group was housed in cages with limited nesting and bedding materials. Briefly, the floor of the ELA cage was covered with pelleted corncob to minimize ammonia levels. A plastic-coated wire mesh (approximately 0.2 cm in diameter) was installed 2.5 cm above the cage floor and half a piece of pressed cotton square was provided for nesting material. The control group was housed in cages with normal nesting and bedding materials as described above. The ELA paradigm lasted for 7 days (P2–9), during which maternal behaviors were recorded three times a day (9:00 a.m. and 2:00 p.m., 7:00 p.m.). The activities of dams were observed for 30 min at each time point, during which dam-pup interaction was scored every 1 min, resulting in thirty 1-min epochs. Following the ELA condition, dams and pups were returned to normal cages.
Tissue sampling and treatment

Mice were weighed at the same time (08:00–10:30) on P2, P9, P21, and P90. At the end of behavior testing, mice were anesthetized with sodium pentobarbital (80 mg/kg) and then perfused via the ascending aorta with 0.9% saline (2 min) and 4% paraformaldehyde in 0.1 M phosphate buffer (PB) (15–20 min). The brains were postfixed in the same fixative for 4–6 h and blocked into two hemispheres. One hemisphere was embedded in paraffin and the other was cryoprotected in 30% sucrose in 0.1 M PB (4 °C). The paraffin tissue blocks were sectioned coronally at 7 μm and corresponding sections from the dorsal striatum (AP 1.42 mm to −0.82 mm) and dorsal hippocampus (AP −1.20 mm to −2.20 mm) of two groups were mounted on one slide, with every tenth section being selected. The cryoprotected tissue blocks were sectioned coronally (20 μm) and every forth section (1 in 4th) was used for fluorescent immunostaining. Adjacent sections were stained with cresyl violet solution or 4',6-diamidino-2-phenylindole (DAPI) to identify the cellular architecture.

Lever-press training and devaluation testing

The training was performed in noise attenuated operant chambers (Shanghai Xinruan Information Technology Co., Ltd.). Before the training, mice were subjected to a food deprivation schedule to maintain approximately 85% of their free-feeding weight (Rossi and Yin, 2012). During the training, lever presses were rewarded with sucrose-containing pellets. Grain-only pellets were used as a sensory-specific control for satiety. The use of a lever and reinforcer (sucrose-containing pellets) in each group was counterbalanced. Mice were conducted a 30-min magazine training session, in which one reinforcer was randomly delivered every 60-sec, ending when 30 reinforcers were earned, followed by training to gain a specific reinforcer by pressing the lever. The lever-press training began with a continuous reinforcement session (2 days) followed by random ratio training, including ratio-5 (RR-5, average one reinforcer every 5 lever presses; 2 days), RR-10 (3 days), and RR-20 (7 days). Times of lever pressing and reinforcer delivery were recorded during daily training. Habitual behavior was measured via a devalued probe test (Rossi and Yin, 2012; O’Hare et al., 2016). An early devaluation test (Test 1) was conducted after the first day of RR-20, and the devaluation test (Test 2) was performed 24 hours after the last RR-20 training. The test was performed based on a selective satiety strategy. In brief, mice were first given open access to one reinforcer (1.4 g) for 1 h in its home cage without bedding. Following the pre-feeding, the animal was subjected to a 5-min devaluation probe test, during which the lever could be pressed, but no reinforcer would be delivered. The number of lever presses was recorded. Mice that didn’t consume 1.4 g of reinforcer were excluded in data analysis. The experiments were performed with the experimenter blind to the training schedule.

Spatial memory testing

Spatial memory function was tested via a novel object location task as described previously (Xu et al., 2018). Prior to training, mice were handled for 2 min and then familiarized with the experimental apparatus for 10 min per day in the absence of objects, for 5 days total. In the training phase, two identical objects were presented in different locations in the apparatus for 10 min of exploration. Testing was conducted 2 h and 24 h after the last training to detect the short-term and long-term memory of trained mice, respectively. In the testing session, object exploration (in one novel and one familiar location) was performed for 5 min. The training and testing were conducted without knowledge of groups and were recorded with a video tracking system. Exploration was scored when a mouse’s head was oriented toward the object within 1 cm or when the nose was touching the object. Exploration times of the object located in the familiar place as well as in the novel place were recorded, and the ratio of time in the novel versus time in the familiar location was used as an index of memory function.

Enzyme-linked immunoassay (ELISA)

The level of serum corticosterone (CORT) was detected via an ELISA kit (Shanghai Enzyme-linked Biotechnology Co., Ltd.). Prior to the collection of blood samples, mice were kept in their home cages without extra disturbance for at least 24 h. On P9, P21, and P90, eyeball blood was quickly collected (within 5 min of disturbance) at the same time (08:30–10:30) from a subgroup of animals. The blood samples were coagulated naturally (2 h, room temperature) and then centrifuged at 3000 rpm for 20 min. The supernatant was carefully collected, and protein concentration was measured according to the instructions. The incubation of sample diluent and enzyme-labeled reagent was performed at 37 °C for 1 h. The reaction was developed at 37 °C for 15 min in the dark and the OD value was read at 450 nm.

Immunohistochemistry (IHC) and immunofluorescence (IF)

The immunostainings were performed as described previously (He et al., 2021). Briefly, the mounted paraffin sections were first deparaffinized in xylene and then hydrated. Sections were treated in 0.3% H₂O₂ in methanol for 30 min to quench endogenous peroxidase activity, followed by blockage of nonspecific binding with 5% normal horse serum in 0.3% Triton X-100 (PBS-T, pH 7.4) for 30 min. The sections were then incubated with a mouse monoclonal anti-PSD-95 (1:8,000, clone 7E3-1B8, Affinity BioReagents) in PBS-T containing 1% BSA for 3 days at 4 °C. After incubation in biotinylated horse-anti-mouse IgG (1:400, Vector; 2 h) and avidin–biotin-peroxidase complex (1:200, Vector; 3 h), the reaction product was visualized by incubating the sections for 10 min in 3,3'-diaminobenzidine (DAB) containing H₂O₂ (Bioenno Tech). IF labeling of PSD-95 was performed on cryopro-
Quantification of PSD-95 immunoreactive (ir) puncta

PSD-95-ir puncta were analyzed via two approaches as described previously (Xu et al., 2017; Xu et al., 2019). (1) Sections from IHC were subjected to the measurement of optical density (OD) of immunoreactivity based on unbiased stereological sampling (West, 1999). The OD value was calculated based on the average value from three fields containing regions of interest using ImageJ (v2). 9–10 sections containing the dorsal striatum or dorsal hippocampus per animal were counted. The OD of the corpus callosum served as the background. All measurements were conducted under the same optical and lighting conditions. (2) Sections from IF staining were used for counting all individual puncta in the region of interest via stereological principles (West, 1999; Xu et al., 2019). 7–8 sections per animal were used and z-stack images were taken at 63×/1.4 using a Zeiss 510 confocal microscope or a Leica microscope (DM6000). A sampling grid of 200 × 200 μm, a counting frame of 25 × 25 μm, and a guard zone of 10 μm were used (Xu et al., 2019). Three-dimensional image stacks were processed for iterative deconvolution at 99% confidence (Velocity 6.3) and subjected to analyses of puncta number and size distribution. Numbers of labeled puncta (per 75 × 75 × 4 μm) from each section were averaged to obtain a value for each brain. Sections from two groups were processed concurrently and analyzed without knowledge of treatment group.

Golgi staining

A cohort of adult mice including both controls and ELA ones (4-month-old, n = 8 mice per group) was subjected to a Golgi staining to label the dendritic spines of neurons in the dorsal striatum and dorsal hippocampus (He et al., 2021; Xu et al., 2022). Animals were perfused via the aorta with 0.9% saline solution for 2 min to flush out blood. The brains were subjected to the staining using a superGolgi kit (Bioenno Tech LLC, Santa Ana, CA, USA). In brief, after 2 days of impregnation in a provided Golgi-Cox solution (22 ± 1 °C), the solution was refreshed and the impregnation was continued for another 7 days. The brains were sectioned coronally at 200 μm (Leica VT1200). Series sections from the dorsal striatum (every other, five sections per brain, AP 1.42 mm to −0.82 mm) and all sections from the dorsal hippocampus (five sections/brain, AP −1.20 mm to −2.20 mm) were mounted on gelatin-coated slides. The staining (15 min, 22 ± 1 °C) and post-staining (15 min, 22 ± 1 °C) were performed in a parallel manner.

Quantitative analyses of dendritic spines

Dendritic spines were analyzed with the aid of Stereo Investigator (MBF Bioscience, Williston, VT, USA). Images were captured with a Nikon E400 equipped with a CCD camera (DS-Fi3) and motorized stage. The number and type of dendritic spines in Golgi-stained sections were evaluated using a stereological fractionator method (Xu et al., 2022). DMS and DLS in the dorsal striatum and strata oriens (SO), radiatum (SR), and lacunosum-moleculare (SLM) in area CA1 were defined using a 5× objective. Spines were counted using a 100×/1.4 oil objective. High-magnification images permitted all spines of a given dendritic segment to be visualized. Spines were classified by size and shape as thin, mushroom-type, and stubby spines (He et al., 2021; Xu et al., 2022). Stubby spines were not included in the analysis, because they were occasionally observed in DMS, DLS, and area CA1. For the stereological analysis, a counting frame of 25 × 25 μm was selected. The sampling grid was 200 × 200 μm and the disector height was 50 μm.

Statistical analysis

Data were analyzed using Prism 9 (GraphPad, San Diego, CA) or SPSS 22.0 (SPSS Inc.). Two-way repeated measures ANOVA was used to compare the dam-pup interactions, body weight gains, CORT levels, and memory performances with group and day/hour as factors, followed by Bonferroni’s post hoc test. Ordinary two-way ANOVA was also employed to detect the differences in PSD-95 puncta and dendritic spines with treatment and region/size/subtype as factors, followed by Sidak’s or Bonferroni’s post hoc test. Student’s t test was used to analyze the difference of total puncta and total spines between ELA mice and controls. Pearson’s test was used for the correlation analysis. The Kolmogorov-Smirnov test was used to test the normality of data sets. When normal distribution could not be assumed, data were log transformed. When sphericity could not be assumed, the Geisser-Greenhouse correction was used. Significance was set at 95% confidence.

RESULTS

Altered cage environment affects the nesting behaviors of dams

Dams reared in cages with limited nesting and bedding materials exhibited altered maternal care behaviors compared to dams in the control condition. During postnatal days 2–9 (P2–9), maternal behaviors including dam-pup interactions and time spent on the nest were quantified. Duration of dam-pup contact as well as time away from the nest area (off nest) were analyzed in three phases including two light phases (a.m. & p.m.) and one dark phase (Fig. 1). Unlike control dams, dams exposed to the altered cage environment (ACE) frequently left their pups, having an increased frequency of nest entries and exits. The ACE dams had a higher number of nest exits (n = 10) compared with control
Altered cage environment creates an adversity condition, interfering offspring development.

Body weight of mice in both groups increased gradually from P2 to P90. Compared with age-matched controls, pups reared under the altered cage environment had a lower body weight gain when they were at ages P21, P56, and P90 (two-way RM ANOVA, $F_{1,14} = 165.2$, $P < 0.0001$, $n = 8$; Bonferroni’s post hoc test, $**P < 0.01$) (Fig. 2A). These pups had a higher level of serum corticosterone (CORT) versus controls at P9 and P21 (two-way RM ANOVA, $F_{1,10} = 29.99$, $P = 0.0003$, $n = 6$; Bonferroni’s post hoc test, $*P < 0.05$), but not at P90 ($P = 0.3$) (Fig. 2B), indicating a stressful state during the critical period of development.

ELA disrupts decision making and spatial memory

Two cohorts of young adult mice (4-month-old) were employed to evaluate the effects of ELA on memory performances. The first cohort was subjected to a striatal-dependent decision-making task (Fig. 3A–C). ELA mice ($n = 9$) and controls ($n = 11$) were trained to press levers to acquire a goal-directed reward of pellets. The rate of lever-pressing increased gradually throughout the training (two-way RM ANOVA, $F_{13,234} = 44.83$, $P < 0.0001$) with a group difference (two-way RM ANOVA, $F_{1,18} = 9.37$, $P = 0.0067$; Bonferroni’s post hoc test, $P > 0.05$) (Fig. 3A). The amount of reinforcer consumed during two tests was similar in both groups ($t_{18} = 1.39$ and 0.66 for Test 1 and Test 2, respectively; $P > 0.05$) (Fig. 3B). In an early devaluation test (Test 1), rate of lever-pressing significantly decreased in both groups of mice when the valued outcome was devalued by sensory-specific satiety (devalued condition) ($F_{1,18} = 118.5$, $P < 0.0001$; Bonferroni’s post hoc test, $**P < 0.01$). However, in a late devaluation test (Test 2), the actions of ELA mice became insensitive to the expected value of the outcome. Specifically, while control mice exhibited decreased rates of lever-pressing at the devalued condition ($F_{1,18} = 96.83$, $P < 0.0001$; Bonferroni’s post hoc test, $**P < 0.01$), ELA mice pressed the levers habitually and were insensitive to outcome devaluation (Bonferroni’s post hoc test, $P = 0.28$). These data showed that ELA mice gradually transferred their goal-directed actions to habitual action as training progressed.

The second cohort was tested in an object location task to evaluate the impacts of ELA on hippocampal-dependent spatial memory. In the testing phase of the object location task (Fig. 3D, E), control mice ($n = 10$) explored the object in a novel location more than the object left in the familiar location both 2 h and 24 h after the training phase, whereas ELA mice ($n = 13$) explored the two objects almost equally (one-sample $t$-test, $t_{12} = 0.05$ and 1.53, $P = 0.96$ and 0.15 for 2 h and 12 h, respectively) and had a lower novel/familiar ratio compared with controls (two-way RM ANOVA, $F_{1,21} = 80.75$, $P < 0.0001$; Bonferroni’s post hoc test, $**P < 0.01$), suggesting defective memory function in the ELA mice. It is noted that differences were not detected in total exploration time during the training ($t_{21} = 0.86$, $P = 0.40$) and testing phases (two-way RM ANOVA, $F_{1,21} = 0.87$, $P = 0.36$), indicating that the reduced ability to remember the location of an object was not a result of residual effects of ELA on general features of behavior.

Alteration of synaptic contacts in the dorsal striatum and dorsal hippocampus of ELA mice

To investigate the synaptic basis of altered cognitive function, brain tissues were harvested at the end of
behavioral testing and subjected to immunostaining for PSD-95, because PSD-95 is present in the majority of excitatory synapses and immunolabeled PSD-95 puncta have been used to represent mature synapses (e.g., Chen et al., 2013; Maras et al., 2014; Xu et al., 2018). The optical density (OD) of PSD-95 immunoreactivity was first stereologically measured, followed by quantitative analysis of the number and sizes of fluorescently labeled PSD-95 puncta/synapses.

In the dorsal striatum, an increased OD value of PSD-95 immunoreactivity was observed in DLS, but not in DMS of ELA mice when compared with controls (two-way ANOVA, $F_{1,32} = 4.36, P = 0.04$; Sidak’s post hoc test, **$P < 0.01$) (Fig. 4A–C, G). However, a decreased OD value was observed in the hippocampus in ELA mice versus controls (two-way ANOVA, $F_{1,48} = 8.33, P = 0.005$), specifically in the stratum radiatum (SR) of area CA1 (post hoc test, **$P < 0.01$) (Fig. 4D–F, H). Focusing on DLS and CA1 radiatum, fluorescently labeled PSD-95 puncta were further quantitatively analyzed (Fig. 5). In DLS (Fig. 5A–E), an increased number of total PSD-95 puncta was observed in ELA mice versus controls ($t_{16} = 4.99, **P < 0.01$). When these puncta were categorized into small ($0.20–0.40 \mu m^3$), and large ($>0.40 \mu m^3$) subgroups (Xu et al., 2018), the increase was significant in both small and large synapses (two-way ANOVA, $F_{1,48} = 25.68, P < 0.0001$; Sidak’s post hoc test, $^*P < 0.05$, **$P < 0.01$). No difference was observed in medium ones ($0.20–0.40 \mu m^3$), and large ($>0.40 \mu m^3$) subgroups (two-way ANOVA, $F_{3,48} = 4.22, P = 0.02$). In CA1 radiatum (Fig. 5F–J), a reduced number of total PSD-95 synapses was observed in ELA mice versus controls ($t_{16} = 5.19, **P < 0.01$), consistent with previous reports of stress-provoked loss of dendritic spines in this region (Chen et al., 2008). Particularly, loss of small synapses was apparent in ELA vs. controls ($F_{1,48} = 36.09, P < 0.0001$; Sidak’s post hoc test, **$P < 0.01$). No difference was observed on medium and large synapses ($P > 0.05$). Taken together, the data from two different approaches suggested that ELA interferes with the development of neuronal circuits differently, resulting in altered synaptic contacts in the DLS and hippocampus.

The effects of ELA on dendritic spines in the dorsal striatum and dorsal hippocampus

The dendritic spines that harbor PSD-95 were further investigated in ELA mice and their controls ($n = 8$). Spines on neurons in the dorsal striatum (DMS and DLS) and area CA1 of the dorsal hippocampus were counted using an unbiased stereological fractionator method (Fig. 6). In the dorsal striatum, an increased number of total spines was apparent in DLS of ELA mice versus controls ($t_{14} = 3.55, **P < 0.01$). No difference was observed in DMS of ELA mice versus controls ($t_{14} = 0.22, P = 0.83$). The spines are classified into thin, mushroom-type, and stubby spines (He et al., 2021; Xu et al., 2022). Stubby spines were seldomly observed and not included in data analysis. In DLS, ELA provoked increase was detected on both thin and mushroom-type spines (two-way ANOVA, $F_{1,28} = 15.70, P = 0.0005$; Sidak’s post hoc test, $^*P < 0.05$). No difference was detected on spine subtypes in DMS ($F_{1,28} = 0.06, P = 0.81$).

In area CA1, the spines were counted in strata oriens (SO), radiatum (SR), and lacunosum-moleculare (SLM). Decreased total spines were apparent in SR ($t_{14} = 3.25, **P < 0.01$) and a tendency of decreased total spines was observed in SO ($t_{14} = 2.01, P = 0.06$) in ELA mice versus controls. Two-way ANOVA analyses revealed an ELA-provoked loss of thin spines in SR ($F_{1,28} = 10.08, P = 0.0036$; Sidak’s post hoc test, **$P < 0.01$) and SO ($F_{1,28} = 4.53, P = 0.04$; post hoc test, $^*P < 0.05$). No group difference was detected on total spines ($t_{14} = 0.38, P = 0.70$) and subtypes of spines ($F_{1,28} = 0.20, P = 0.65$) in SLM. These data suggest that ELA-provoked changes in synaptic contacts can be detected on dendritic spines in DLS and area CA1.

Correlation analysis of PSD-95 puncta/synapses and cognitive functions

To explore whether altered synaptic contacts in DLS and CA1 radiatum contribute to habit and spatial memory functions, respectively, the correlation between synapses and memory performances was analyzed. The number of total synapses in DLS was significantly correlated with the lever-pressing in the devalued condition versus the valued condition (devalued/valued ratio) (Pearson $r = 0.73, P < 0.0005$; Fig. 7A). When the synapses were classified into small ($<0.20 \mu m^3$), medium ($0.20–0.40 \mu m^3$), and large ($>0.40 \mu m^3$) subgroups (Fig. 7B), a positive correlation was detected between the small synapses and performance in the

Fig. 2. The impacts of rearing in altered cage environment during P2–9 on offspring. (A) Pups that experienced early-life adversity (ELA) created by an altered cage environment had a lower body weight gain during development and as young adults compared with age-matched controls (two-way RM ANOVA, $F_{1,14} = 166.2, P < 0.0001, n = 8$). A significant difference was observed at P21, P56, and P90 (Bonferroni’s post hoc test, **$P < 0.01$). (B) Increased levels of serum corticosterone (CORT) were observed in offspring exposed to ELA compared with age-matched controls when they were at ages P9 and P21 (two-way RM ANOVA, $F_{1,10} = 29.99, P = 0.0003$; Bonferroni’s post hoc test, $^*P < 0.05$).
Early-life adversity (ELA) affects habit memory (A–C) and spatial memory (D, E). (A–C) ELA mice were not sensitive to outcome devaluation in a lever-pressing task. (A) Rate of lever presses in mice that experienced ELA at P2–9 compared with controls (two-way RM ANOVA, $F_{1,18} = 9.37, P = 0.0067$; Bonferroni’s post hoc test, $P < 0.05$; $n = 9–11$). The training lasted 14 days, consisting of 2 days of continuous reinforcement (ratio-1, one reinforcer per lever press) and 12 days of increasing random ratio schedules of reinforcement (ratio-5, 10, or 20, one reinforcer every 5, 10, or 20 lever presses, respectively). Arrows denote the devaluation tests performed early (Test 1) and late (Test 2). (B) No difference was observed in the amount of reinforcer consumed in an early (Test 1, $t_{18} = 1.39, P > 0.05$) and a late test (Test 2, $t_{18} = 0.66, P > 0.05$). (C) ELA mice became insensitive to the expected value of the outcome when they were tested at the end of the training (Test 2) (Bonferroni’s post hoc test, $P = 0.28$), but not at Test 1 ($F_{1,18} = 118.5, P < 0.0001$; Bonferroni’s post hoc test, **$P < 0.01$). The controls had a good devaluation action at Test 1 and Test 2 ($F_{1,18} = 118.5$ and 96.83, respectively; **$P < 0.01$). (D, E) Spatial memory was detected via a novel object location task. After a 5-day habituation, mice were trained for 10 min with two identical objects located at two different locations. Short- and long-term spatial memories were tested at 2 h and 24 h later, respectively. During the 5-min testing, one object was moved to a novel location. The ratio of time in novel location vs. familiar location was used as an index to represent spatial memory. There were no significant differences in total exploration time between the two groups (D) during training ($t_{13} = 0.86, P = 0.40$) and testing (two-way RM ANOVA, $F_{1,21} = 0.87, P = 0.36; n = 10–13$). (E) ELA mice had a lower novel/familiar ratio both 2 h and 24 h after the training compared with controls (two-way RM ANOVA, $F_{1,21} = 80.75, P < 0.0001$; Bonferroni’s post hoc test, **$P < 0.01$).

Strong correlation was also found in the group of large synapses ($r = 0.69, P = 0.0004$), but not in the group of medium ones ($r = 0.34, P = 0.1692$).

The number of total synapses in CA1 radiatum was correlated with the novel/familiar ratio in the novel location task (Pearson $r = 0.21, P = 0.0077$)

DISCUSSION

Adverse experiences during a sensitive period in early postnatal days have been associated with altered neural differentiation and increased risk for cognitive deficits across the lifespan. Indeed, stress in early life disturbs the maturation of dendritic spines and shapes the underlying synaptic contacts, fundamentally influencing the development of brain structure and function (Chen and Baram, 2016; Birn et al., 2017). Here, we generate an ELA mouse model via limited nesting and bedding materials during P2–9 and find that the altered cage environment interrupts maternal nesting care, which is consistent with previous reports and has been termed as fragmented or unpredictable maternal care (Ivy et al., 2008; Rice et al., 2008; Bolton et al., 2019). Importantly, pups under the altered maternal care have persistent high levels of serum CORT, indicating a chronic stress state. To investigate the impacts of ELA on behavioral outcomes in adulthood, ELA mice have been subjected to striatal-dependent lever-pressing training and devaluation testing as well as a hippocampal-dependent object location task. The results suggest an enhanced habit memory, but damaged spatial memory in ELA mice versus age-matched controls. Furthermore, selective changes of synapses and dendritic spines are observed in DLS and specific layers of area CA1. Correlation analysis reveals that the increased small and large synapses in DLS might drive
ELA mice to make decisions mostly relying on habit memory, whereas loss of small synapses in area CA1 leads to defective spatial memory in ELA mice.

In the current study, the maternal nesting behaviors have been measured and scored in three specific time periods, which were typically employed in previous investigations (Ivy et al., 2008; Rice et al., 2008; Bolton et al., 2019; Gallo et al., 2019). We did not continuously record nesting behaviors throughout the whole circadian cycle. Continuous video recordings of the home cage would be helpful for capturing more detailed maternal behaviors (Gallo et al., 2019). Here, maternal behaviors have been observed over the course of a 30-min period rather than every other minute over 30 min (Rice et al., 2008; He et al., 2021). The resulting 30-min epochs of recording reflect the nesting behaviors of dams more completely. We found that dams reared in limited nesting and bedding materials during postnatal days 2–9 often left the nest area and had an increased frequency of nest entries and exits, which is consistent with previous reports. Furthermore, we found that pups exposed to an altered cage environment had a lower body weight gain, which persists into adulthood. These pups also displayed an upregulated basal level of serum CORT. These data suggest that the altered cage environment provokes changes in dam-pup interactions, inducing stress in the pups and leading to potential consequences for offspring development.

During the critical period of development, adverse experiences can produce life-long deficits in memory processing and motivational behavior across many species (e.g., Vivinetto et al., 2013; Chen and Baram, 2016; Tzanoulinou and Sandi, 2017; Stuart et al., 2019; Chistiakov and Chekhonin, 2019; Marrocco et al., 2019; Miguel et al., 2019; Schmeck, 2020; Rocha et al., 2021). Studies from rodents have shown that adverse experiences often exert a particularly strong influence in shaping the functional properties of an immature brain. It has been reported that the hippocampus and several motivational brain regions appear to be particularly vulnerable to stress and stress-related molecules (Joëls and Baram, 2009; Chen et al., 2012; Sandi and Haller, 2015). While the negative impacts of early-life stress on hippocampal-dependent memory have been documented in numerous studies, loss of dendritic spines, impoverished dendritic branches, and reduced hippocampal volume have been found in adult mice exposed to chronic early-life stress (Brunson et al, 2005; Molet et al, 2014). Considering the functional complexity of the dorsal versus ventral hippocampus (e.g., Moser et al., 1993; Kjelstrup et al., 2008; Bannerman et al., 2014) as well as unclear neuronal connections between the ventral hippocampus and dorsal striatum, we focused on the dorsal hippocampus. In this study, ELA-provoked loss of PSD-95-ir puncta was primarily observed in the stratum radiatum of area CA1, which is supported by the loss of thin spines in this layer. It is known that CA1 radiatum is full of numerous oblique dendritic branches of pyramidal
cells. These branches carry a large number of spines with postsynaptic density (PSD). Quantitative analysis has shown that these branches constitute the majority of the dendritic tree surface (Horner et al., 1991; Megías et al., 2001) and provide the main target of excitatory synaptic inputs including the Schaffer axon terminals (Megías et al., 2001; Lavenex et al., 2007). It is well known that the Schaffer pathway is crucial for hippocampal-dependent spatial navigation (Moser et al., 1993; Muller and Stead, 1996). Thus, loss of spines and PSD-95-ir puncta in this area may eliminate the chance of forming synaptic contacts, deteriorating synaptic plasticity and spatial memory function. It is interesting to note that the effects of ELA on PSD-95-ir puncta are mainly restricted to small synapses. Further studies are needed to understand the basis responsible for the preferential loss of small synapses.

The negative impacts of ELA on spatial memory and hippocampal neurons have been extensively studied. However, the effects of ELA on striatal structure and function largely remain unknown. The dorsal striatum is not a functionally uniform region. This large and heterogeneous structure can be subdivided into medial (DMS, corresponds to the caudate nucleus in human) and lateral (DLS, analogous to the putamen) subdivisions. Emerging evidence suggests that the dorsal striatum may serve as a principal interface implicated in motor coordination, reward circuitry, and addictive action (Scimeca and Badre, 2012; Yager et al., 2015; Ferbinteanu, 2016; Lipton et al., 2019). Specifically, DMS is heavily involved in goal-directed action learning and performance (Yin et al., 2005), while DLS is necessary and critical for habitual action learning and performance (Yin and Knowlton, 2006). Clinical data have shown that exposure to chronic stress can bias decision-making strategies in humans toward habits via reorganizing the corticostriatal circuits (Starcke and Brand, 2012; Ferreira et al., 2019). Data from adult rodents have provided further evidence to show that stressed adult animals become insensitive to changes in outcome value and resistant to changes in action-outcome contingency (Dias-Ferreira et al., 2009). We report here that young adult mice that experienced ELA gradually transfer their goal-directed actions to habitual action as training progresses. These ELA mice become insensitive to outcome devaluation and pressed the levers habitually in a devaluation test. In the present study, mice were food deprived before...
the training and testing. Doing so drives them to press the lever for a food reward, because food deprivation motivates animals to find and consume food (Rossi and Yin, 2012; Chen et al., 2016). However, food deprivation leads to an adaptive reduction in basal metabolic rate, which may exert a profound effect on goal-directed behavior, particularly when the reward is driven by food-related cues. Indeed, food deprivation affects psychophysiological responses to a broad range of affective and food-related cues in both humans and animals. It has been reported that food deprivation strongly increases the activity of neurons in the structures involved in affective valuation and decision making (Moscarello et al., 2009). It is unknown whether food deprivation impacts dorsal striatum associated decision making. To minimize the potential effects of food deprivation on behavioral outcomes, mice in the present study were subjected to a feeding schedule to maintain approximately 85% of their free-feeding weight, which is a commonly used strategy in the literatures (e.g., Yin et al., 2006; Rossi and Yin, 2012; O’Hare et al., 2016).

To explore the synaptic basis of the preferential influence of ELA on habit memory, excitatory synapses in the dorsal striatum have been immunolabelled with PSD-95 as described above. The scaffolding protein PSD-95 is uniformly distributed across the full extent of the synaptic active zone. Because PSD-95 immunolabelling corresponds well with the size of postsynaptic density (Aoki et al., 2001), PSD-95-ir puncta have been quantified to represent the number and size of excitatory synapses (Chen et al., 2013; Maras et al., 2014; Xu et al., 2018). Here, we found that ELA mice have increased expression of PSD-95 in DLS, but not DMS. The spine data support this region-dependent increase of synaptic contacts in mice that experienced ELA. Specifically, both thin and mushroom-type spines are increased in DLS, while loss of thin spines are observed in area CA1 in ELA mice. The selective augmentation of synaptic contacts in DLS positively correlates with the enhanced habit memory in ELA mice. Increased PSD-95 synapses in DLS may be a direct response of this structure to ELA, or may derive from altered hippocampal inputs to...
this structure, because the dorsal striatum and hippocampus can dynamically cooperate (Ferbinteanu, 2016; Gasser et al., 2020; van de Ven et al., 2020). Further studies are required to understand the interaction between striatal cells and hippocampal neurons.

In summary, the data presented here demonstrate measurable impacts of ELA on memory functions in adulthood. While ELA has a detrimental effect on spatial memory, adversity during postnatal days 2–9 shifts goal-directed action to habitual action. The altered memory performances correlate with the synaptic contacts in the corresponding brain areas. Increased synapses in selective areas of the dorsal striatum might drive ELA mice to make decisions largely relying on habit memory. However, the reduced synaptic contacts in hippocampal CA1 contribute to defective spatial memory in ELA mice.

CONFLICTS OF INTEREST
None.

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