PSD-95 in CA1 Area Regulates Spatial Choice Depending on Age

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Cognitive processes that require spatial information rely on synaptic plasticity in the dorsal CA1 area (dCA1) of the hippocampus. Since the function of the hippocampus is impaired in aged individuals, it remains unknown how aged animals make spatial choices. Here, we used IntelliCage to study behavioral processes that support spatial choices of aged female mice living in a group. As a proxy of training-induced synaptic plasticity, we analyzed the morphology of dendritic spines and the expression of a synaptic scaffold protein, PSD-95. We observed that spatial choice training in young adult mice induced correlated shrinkage of dendritic spines and downregulation of PSD-95 in dCA1. Moreover, long-term depletion of PSD-95 by shRNA in dCA1 limited correct choices to a reward corner, while reward preference was intact. In contrast, old mice used behavioral strategies characterized by an increased tendency for perseverative visits and social interactions. This strategy resulted in a robust preference for the reward corner during the spatial choice task. Moreover, training decreased the correlation between PSD-95 expression and the size of dendritic spines. Furthermore, PSD-95 depletion did not impair place choice or reward preference in old mice. Thus, our data indicate that while young mice require PSD-95-dependent synaptic plasticity in dCA1 to make correct spatial choices, old animals observe cage mates and stick to a preferred corner to seek the reward. This strategy is resistant to the depletion of PSD-95 in the CA1 area. Overall, our study demonstrates that aged mice combine alternative behavioral and molecular strategies to approach and consume rewards in a complex environment.

Key words: aging; CA1; dendritic spines; PSD-95; social interactions; spatial choice

Significance Statement

It remains poorly understood how aging affects behavioral and molecular processes that support cognitive functions. It is, however, essential to understand these processes to develop therapeutic interventions that support successful cognitive aging. Our data indicate that while young mice require PSD-95-dependent synaptic plasticity in dCA1 to make correct spatial choices (i.e., choices that require spatial information), old animals observe cage mates and stick to a preferred corner to seek the reward. This strategy is resistant to the depletion of PSD-95 in the CA1 area. Overall, our study demonstrates that aged mice combine alternative behavioral and molecular strategies to approach and consume rewards in a complex environment. Second, the contribution of PSD-95-dependent synaptic functions in spatial choice changes with age.

Introduction

Many cognitive functions deteriorate with age, particularly, hippocampus-dependent cognitive processes that require spatial

information (Hedden and Gabrieli, 2004; Kennard and Woodruff-Pak, 2011; Tromp et al., 2015). However, older people and animals are still able to use spatial information to direct their

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behavior (Kiryk et al., 2011; Aziz et al., 2019; Kwapis et al., 2019; Śliwińska et al., 2020). Aged individuals often use alternative behavioral strategies for spatial tasks (Bach et al., 1999) and require more training trials (Gallagher et al., 1993; Murphy et al., 2004), and the spatial memories are more resistant to updating (Jones et al., 2015; Aziz et al., 2019), altogether suggesting alternative neuronal mechanisms.

NMDA receptor-dependent Hebbian forms of synaptic plasticity are believed to be fundamental mechanisms underlying hippocampus-dependent cognitive processes (Malenka and Bear, 2004; Kessels and Malinow, 2009). Most studies to date have linked cognitive functions with long-term potentiation (LTP) of synaptic transmission (Bliss and Collingridge, 1993; Morris et al., 2003). However, accumulating data indicate that some cognitive processes may also rely on long-term depression (LTD) of synaptic transmission (Kemp and Manahan-Vaughan, 2007; Connor and Wang, 2016). Accordingly, several studies have linked impairments of LTP and LTD with decreased ability to form hippocampus-dependent memories in aged animals (Bach et al., 1999; Luo et al., 2015; Temido-Ferreira et al., 2020). Meanwhile, alternative synaptic processes, such as the formation of multi-innervated dendritic spines (Aziz et al., 2019) and NMDAR-independent LTD (Lee et al., 2005), have been documented during memory formation in aged animals. How aging affects behavioral and molecular processes that support cognitive functions remains poorly understood. Understanding these processes is required to develop therapeutic interventions to support successful cognitive aging.

Accumulating data indicate that the dorsal CA1 area (dCA1) is a critical structure that enables spatial choice (i.e., choice that uses spatial information to suppress inappropriate behaviors; Bannerman et al., 2012, 2014). The current study aims to identify how aging affects behavioral strategies and synaptic processes in the dCA1 that support spatial choices. To this end, we used an IntelliCage setup as it provides close-to-ecological conditions to investigate spatial memory and spatial choice (Kiryk et al., 2011; Śliwińska et al., 2020), nonspatial aspects of reward-driven behavior (Radwanska and Kaczmarek, 2012), and social interactions (Kiryk et al., 2011; Harda et al., 2018). To study synaptic plasticity induced by the training, we applied *ex vivo* analysis of dendritic spines in the dCA1 of *Thy1-GFP(M)* transgenic mice (Feng et al., 2000) combined with PSD-95 protein expression analysis. PSD-95 is a key protein at mature glutamatergic synapses (Aoki et al., 2001; Chen et al., 2011). Its expression is regulated during synaptic plasticity (Steiner et al., 2008; Nowacka et al., 2020) and predicts stability of dendritic spines (El-Husseini et al., 2000; Ehrlich et al., 2007; Murmu et al., 2013; Meyer et al., 2014). Knockdown of PSD-95 has been shown to impair NMDAR-dependent LTD (Ehrlich et al., 2007) as well as spatial learning, conditioned taste aversion, and simple operant associative learning (Migaud et al., 1998; Elkobi et al., 2008; Fitzgerald et al., 2015). However, it is unknown whether PSD-95-dependent synaptic plasticity is affected in the aging brain.

Overall, our data indicate that old mice use social observation and perseverative behaviors to make spatial choices. PSD-95 depletion in the dCA1 did not affect this alternative strategy and affected only the spatial choice in young mice. Thus, our study shows that aged animals combine alternative behavioral and molecular strategies for spatial choice in a complex environment. Second, the contribution of PSD-95-dependent synaptic functions in spatial choice changes with age.

Materials and Methods

Animals. C57BL/6J mice were purchased from the Medical University of Białystok (Białystok, Poland). *Thy1-GFP(M)* mice (*Thy1-GFP^{+/+}*; Feng et al., 2000) were bred as heterozygotes with the C57BL/6J background in the Animal House of the Nencki Institute of Experimental Biology and genotyped as previously described (Feng et al., 2000). Young, adult mice were 5 ± 1 months old during the behavioral training and old individuals were 18 ± 2 months old. Only female mice were used for all experiments, as males are too aggressive for group housing in the IntelliCages (Harda et al., 2018; Śliwińska et al., 2020). All mice were housed with access to food and water *ad libitum* and 12 h dark/light cycle, 23–24°C and 35–45% humidity. The studies were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), Animal Protection Act of Poland, and were approved by the First Local Ethics Committee in Warsaw. All efforts were made to minimize the number of animals used and their suffering.

Place preference and avoidance in IntelliCages. The IntelliCage system (NewBehavior; <https://www.tse-systems.com/>) consists of a large standard rat cage (height, 20.5 cm; 40×58 cm at the top; 55×37.5 cm at the base). In each corner of a cage, a triangular learning chamber is located with two bottles. Only one mouse can enter a corner through a plastic ring (outer ring, 50 mm diameter; inner ring, 30 mm diameter; 20 mm depth into the outer ring) that ends with two holes (diameter, 13 mm) that provide access to two bottle nipples. Each visit to the corner, the nosepoke at the doors governing access to the bottles and lick were recorded by the system and were ascribed to a particular animal. During experiments, two corners were active. Groups of 8–15 mice were trained per IntelliCage.

Under brief isoflurane anesthesia, mice were subcutaneously injected with unique microtransponders (length, 10.9 mm; diameter, 1.6 mm; Slim Microchip T-SL, DATAMARS), which allow for mice identification in the IntelliCage. Next, animals were given 3 d to recover before introduction to the IntelliCage. Experiments consisted of the following two phases: habituation (7–11 d) and training (2 h or 1 d). During habituation, animals had access to water in both cage corners. Four days before training, mice had 24 h access to 5% sucrose solution (in tap water) from the top of the IntelliCage to become familiarized with the taste. Baseline preference to the cage corners was measured during the last day of the habituation as the percentage of visits or licks. During place preference training, water was replaced by 5% sucrose (Sigma-Aldrich) in the less visited corner. During place aversion training, water in the preferred corner was replaced by quinine solution (200 μ M in tap water; Sigma-Aldrich). The change of corner preference during training (percentage of visits to sucrose/quinine corner) was compared with the preference of the same corner during the last day of habituation (Hab.) and used as an index of spatial choice. The change of lick preference was used as an index of nonspatial reward memory. Training started at the beginning of the dark phase (12:00 A.M.).

To measure social interaction in the IntelliCage, we used analysis of immediate visits (i.e., visits of cage mates, with an interval of <1 s; Dzik, 2018; Dzik et al., 2018; Harda et al., 2018; PyMICE software; RRID:SCR_010505). To determine the frequency of patrolling visits, we analyzed the percentage of visits without licks. Perseveration was calculated as the percentage of visits in a preferred corner on the last day of habituation.

Immunostaining on brain slices. Mice were anesthetized and transcardially perfused with filtered PBS (Sigma-Aldrich) followed by 4% PFA (Sigma-Aldrich) in PBS. Brains were removed and placed overnight in the same fixing solution and transferred to 30% sucrose in PBS for 3 d. Next, coronal brain sections (40 μ m thick) were prepared (Leica CM1950 Cryostat, Leica Biosystems) and stored at -20°C in PBSAF [PBS, 15% sucrose (Sigma-Aldrich), 30% ethylene glycol (Sigma-Aldrich), and 0.05% NaN_3 (Sigma-Aldrich)]. The sections were washed with PBS (Sigma-Aldrich) followed by 1 h incubation in a blocking solution (5% normal donkey serum in PBS/0.3% Triton X-100) and overnight incubation with a primary antibody directed against PSD-95 (1:500; catalog #MAB1598, Merck Millipore; RRID:AB_94278). Next, the sections were washed in PBS with 0.3% Triton X-100 and incubated

for 90 min with the secondary antibody: anti-mouse Alexa Fluor 555 (1:500; catalog #A31570, Thermo Fisher Scientific; RRID:AB_2536180). The sections were mounted on glass microscope slides (Thermo Fisher Scientific), air dried, and coverslipped with Fluoromount-G medium with DAPI for fluorescence (catalog #00–4959–52, Thermo Fisher Scientific).

Analysis of dendritic spines and PSD-95(+) puncta. The staining was analyzed with the aid of a confocal, laser-scanning microscope. z-Stacks of dendrites in the medial partition of the stratum radiatum of dCA1 area were acquired using the Zeiss Spinning Disk microscope (63 $\times$ oil-objective, numerical aperture 1.4; pixel size, 0.13 $\times$ 0.13 μ m; Zeiss). A series of 18 continuous optical sections (67.72 $\times$ 67.72 μ m), at 0.26 μ m intervals, were scanned along the z-axis of the tissue section. Six to eight z-stacks of microphotographs were taken per animal, from every sixth section through dCA1 (one dendrite per neuron per image). The z-stacks were flattened to maximal projections and analyzed with Fiji software. The density and area of PSD-95(+) puncta and dendritic spines, as well as their colocalization, were analyzed using ImageJ 1.52n software and measured using the analyze particle tool, as previously described (Fedulov et al., 2007). Each dendritic spine was manually outlined, and the spine area was measured with the measure tool. Custom-written Python scripts were used for ImageJ 1.52n software to analyze the total area of PSD-95(+) puncta per dendritic spine.

Stereotactic intracranial injections. Mice were anesthetized with isoflurane (5% for induction, 1.5–2.0% after) and fixed in the stereotactic frame (catalog #51503, Stoelting), and their body temperatures were maintained using a heating pad. Stereotactic injections were performed bilaterally into the dCA1 region of the hippocampus using coordinates from the bregma, as follows: anteroposterior, -2.1 mm; mediolateral, ± 1.1 mm; dorsoventral, -1.3 mm (according to Paxinos and Franklin, 2001). A total of 0.5 μ l of virus solution was microinjected through a beveled 26 gauge metal needle, attached to a 10 μ l microsyringe (model SGE010RNS, WPI) connected to a microsyringe pump (model UMP3, WPI) and its controller (model Micro4, WPI) at a rate 0.1 μ l/min. The microsyringe was left in place for an additional 10 min following injection to prevent leakage of the vector. Mice were injected with lentiviral vectors (LVs) coding short hairpin RNA (shRNA) silencing PSD-95 expression [H1-shRNA_PSD95-Ub-GFP (0.5 μ l/site, viral titer 2.52×10^8 / μ l); a gift from Oliver M. Schlüter, European Neuroscience Institute, Göttingen, Germany; Schlüter et al., 2006] or a control vector based on a pSUPER shRNA targeting the Renilla luciferase (5'-CTGACGCGGAATACTTCGA-3') cloned into pTRIP (H1-shRNA_luciferase; 0.5 μ l/site; viral titer, 6.52×10^8 / μ l) were used. The viruses were prepared by the Laboratory of Animal Models at Nencki Institute of Experimental Biology, Polish Academy of Sciences. After the surgery, animals were given 14 d to recover before training in the IntelliCages. After training, the animals were perfused with 4% PFA in PBS, and brain sections from the dorsal hippocampus were immunostained for PSD-95 and imaged with a Zeiss Spinning Disk confocal microscope (magnification, 10 $\times$) to assess the extent of the viral expression and PSD-95 expression.

Experimental design and statistical analysis. Data acquisition and quantification were performed by an experimenter blind to experimental groups. All statistical analyses were performed using GraphPad Prism 8 Software. The exact sample size (the number of mice, dendrites, or spines) of each experiment is provided in figures or figure legends. For data with normal distribution and equal variance, Student's *t* test, one-way or two-way ANOVA, or a mixed-effects model with repeated measures (RM) and *post hoc* Sidak's or Dunn's tests for multiple comparison were used. Least significant difference (LSD) *post hoc* tests were used only for planned comparisons. The Wilcoxon matched-pairs signed-rank test was used for data with nonparametric distributions.

Areas of dendritic spines and PSD-95 puncta did not follow normal distributions and were analyzed with the Kruskal–Wallis test. Frequency distributions of PSD-95 to the spine area ratio were compared with the Kolmogorov–Smirnov test. Correlations were analyzed using Spearman correlation, and the difference between slopes or elevation between linear regression lines was calculated with ANCOVA. All data with normal distribution are presented as the mean $\pm$ SEM. For samples that did not follow a normal distribution, median and interquartile range (IQR)

values are shown. The difference between the experimental groups was considered significant at $p < 0.05$.

Results

Spatial choice training in young and old mice

Young adult (5 ± 1 months old, $n = 6$) and old (18 ± 2 months old, $n = 6$) mice were separately housed in two IntelliCages. During habituation, mice had access to two active corners with water. During training, sucrose replaced water in the corner less visited by mice during habituation (Fig. 1A; Harda et al., 2018; Śliwińska et al., 2020). Activity (number of visits to the corners) and liquid consumption (on the last day of habituation and training) were measured to verify that the old mice did not have major behavioral impairments. There was a significant effect of training and no effect of age on the activity of mice (two-way RM ANOVA, training: $F_{(1,10)} = 33.10$, $p < 0.001$; age: $F_{(1,10)} = 0.094$, $p = 0.766$) and liquid consumption (training: $F_{(1,10)} = 28.12$, $p < 0.001$; age: $F_{(1,10)} = 4.054$, $p = 0.072$). As confirmed by Sidak's multiple-comparisons *post hoc* tests both young and old animals increased activity (young and old, $p < 0.05$; Fig. 1B.i) but only young mice drank more during training (young, $p < 0.001$; old, $p = 0.128$; Fig. 1B.ii), compared with the last day of habituation. Moreover, there was no difference in activity between young and old mice during habituation or training. These observations show that young and old mice do not differ in basal and training-induced activity, which indicates no gross motor impairments in aged mice.

During training, a correct spatial choice was measured as a change of visit preference to a reward corner, while a nonspatial reward memory was measured as a change of lick preference. Both young and old mice increased the frequency of visits to a reward (sucrose) corner (mixed-effects model: $\chi^2 = 19.84$, $df = 1$, $p < 0.001$; effect of training: $F_{(3,076,30.15)} = 50.82$, $p < 0.001$), indicating the formation of a spatial preference (Fig. 1C.i). Old mice showed, however, a greater preference for the reward corner than young mice (effect of age: $F_{(1,10)} = 12.31$, $p = 0.006$; age $\times$ time interaction: $F_{(5,49)} = 5.418$, $p < 0.001$), as confirmed by LSD *post hoc* test for planned comparisons ($p < 0.05$; Fig. 1C.i). Moreover, young, and more so the old mice, increased the frequency of licks at the sucrose corner (mixed-effects model, $\chi^2 = 34.90$, $df = 1$, $p < 0.001$; effect of training: $F_{(1,788,17.16)} = 137.1$, $p < 0.001$), indicating nonspatial reward memory as demonstrated in a significant effect of age ($F_{(1,10)} = 15.36$, $p < 0.003$) and age $\times$ time interaction ($F_{(5,48)} = 8.865$, $p < 0.001$; Fig. 1C.ii). Thus, old mice showed a greater change in reward corner preference than young mice ($p < 0.001$; Fig. 1C.i, C.ii).

Overall, our data indicate that during training, both young and old mice made mostly correct spatial choices and formed nonspatial reward memory. Surprisingly, the change in reward corner preference was more robust in the old mice group. The frequency of visits in the IntelliCage corners is determined not only by the choices that require spatial information but also by habitual patrolling cage corners, visits in preferred places, as well as social interactions (Kiryk et al., 2011, 2020; Harda et al., 2018; Śliwińska et al., 2020). Therefore, we tested whether these innate mouse behaviors affected place preference during training.

The frequency of patrolling visits (visit without licks; Kiryk et al., 2020) decreased significantly during training (two-way RM ANOVA, $F_{(1,10)} = 56.54$, $p < 0.001$), and there was no age effect ($F_{(1,10)} = 3.420$, $p = 0.094$; Fig. 1D). Sidak's *post hoc* tests confirmed no significant difference in the frequency of patrolling visits during habituation and training between young and old

Figure 1. Old mice develop a robust preference for the reward corner as a result of an increased frequency of perseverative and social visits. **A**, Experimental timeline. During Hab., mice had access to two active corners with water. During spatial choice training, sucrose was given in the less visited corner during habituation. Young and old animals were housed separately. **B**, Summary of data showing mice activity during habituation and training. Daily visits (**B.i**) and licks of all liquids (**B.ii**) are shown. **C**, Summarized results show an increase in reward corner preference during training. Change of visit (**C.i**) and lick preference (**C.ii**) from the sucrose corner is shown. A dark phase of a day is indicated as a gray background. **D–F**, Summary data of patrolling visits (without lick; **D**), perseverative visits (**E.i**), and licks (**E.ii**) to the preferred corner, and social visits (following other mice to the corners for intervisit interval < 1 s) during habituation and training (**F**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, for *post hoc* tests. Mean $\pm$ SEM values are shown for **B.i** to **E.ii**; median $\pm$ IQR values are shown for **F**.

mice (both $p > 0.05$). The decrease in the frequency of patrolling visits during training indicates that both young and old mice limited cage exploration and therefore is not the reason for the difference in the place preference scores observed between young and old mice.

Perseveration is measured as the frequency of visits and licks in a preferred corner. During habituation, old mice were more perseverative in visits (Student's t test, $t = 4.194$, $df = 10$, $p = 0.002$; Fig. 1*E.i*) and licks ($t = 4.500$, $df = 10$, $p = 0.001$) compared with the young mice (Fig. 1*E.ii*). Therefore, these perseverative behaviors of the old mice contributed to a stronger reward corner preference during training.

Finally, we analyzed the frequency of social visits [immediate (interval, < 1 s) visits after another mouse; Harda et al., 2018] in young and old mice (Fig. 1*F*). Wilcoxon matched-pairs signed-rank tests showed that during training young animals performed fewer social visits than during habituation ($p = 0.031$), while the

old mice performed more ($p = 0.031$), suggesting that only old mice observed and followed cage mates during the training. Together, our experiment indicated that both young and old mice made correct spatial choices and formed nonspatial memory. Old mice had a greater change in corner preference during the training as a result of the behavioral strategy to increase perseverative and social visits.

The effect of social interactions on spatial choice

To validate the role of social interactions in spatial choice in old mice, we tested whether spatial choice and nonspatial learning of aged mice are altered by the presence of young cage mates. To this end, new cohorts of young ($n = 9$) and old ($n = 13$) mice were mixed in the IntelliCages (Fig. 2*A*) and were trained together according to the protocol described in Figure 1*A*. We observed a significant effect of training ($F_{(1,19)} = 22.17$, $p < 0.001$) but not age (two-way RM ANOVA, $F_{(1,19)} = 3.643$, $p =$

Figure 2. Perseverative spatial choice of old mice is affected by social interactions with young animals. **A**, Experimental timeline. During habituation, mice had access to two active corners with water. During place preference training, sucrose was given in the less visited corner during habituation. Young and old animals were kept together throughout the experiment. **B**, Summary of data showing mice activity during habituation and training. Daily visits (**B.i**) and licks of all liquids (**B.ii**) are shown for a group of old and young mice living in one cage. **C**, Summarized results showing an increase in reward corner preference during training. Data for visits (**C.i**) and licks (**C.ii**) from the sucrose corner are shown. The dark phase of the day is indicated as a gray background. **D–F**, Summary of data showing the frequency of patrolling visits (without licks; **D**), perseverative visits (**E.i**) and licks (**E.ii**) to a preferred corner, and social visits (visits in the corners following another mouse with an intervisit interval of <1 s; **F**) during habituation and training. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, for *post hoc* tests. Mean $\pm$ SEM values are shown.

0.072) on mouse activity measured as daily visits (Fig. 2*B.i*). As revealed by Sidak's *post hoc* tests, young and old mice both increased activity during training ($p = 0.042$ and $p < 0.001$). There was also a significant effect of training ($F_{(1,20)} = 40.01$, $p < 0.001$), but no effect of age ($F_{(1,20)} = 0.496$, $p = 0.489$) on liquid consumption (Fig. 2*B.ii*). As confirmed by *post hoc* analysis, both age groups increased consumption during training ($p < 0.001$ for both). Thus, mixing young and old mice did not affect their activity during training, but slightly increased reward consumption by old mice.

The analysis of reward corner preference during training showed a significant effect of time on the change of visit preference (mixed-effects model, $\chi^2 = 3.342$, $df = 1$, $p = 0.068$; $F_{(3,247,61.69)} = 8.7$, $p < 0.001$), indicating correct spatial choices (Fig. 2*C.i*). Moreover, there was a significant effect of age ($F_{(1,20)} = 12.85$, $p < 0.002$) and interaction between age and time ($F_{(5,95)} = 2.385$, $p = 0.044$). Sidak's multiple-comparisons *post hoc* tests revealed that the difference between

young and old animals was significant only at the beginning of training (0.5 h, $p = 0.005$) and at the end of the dark phase (12 h, $p = 0.001$), but not at other time points. Moreover, the analysis of licks in the reward corner showed a significant effect of training (mixed-effects model, $\chi^2 = 5.734$, $df = 1$, $p = 0.017$; $F_{(3,064,53.93)} = 42.33$, $p < 0.001$), indicating nonspatial memory formation (Fig. 2*C.ii*). There was no effect of age ($F_{(1,20)} = 0.612$, $p = 0.443$) or interaction between age and time ($F_{(5,88)} = 1.235$, $p = 0.300$), demonstrating that young and old mice behaved similarly in a mixed population.

We further asked whether the frequency of patrolling, perseverative, and social visits were changed in a mixed population, compared with separate populations. We found no significant effect of age (two-way RM ANOVA, $F_{(1,20)} = 0.269$, $p = 0.610$) and training ($F_{(1,20)} = 1.943$, $p = 0.179$) on the frequency of patrolling visits (Fig. 2*D*). Thus, unlike separate populations, the frequency of patrolling visits in the mixed population remained high during training. Moreover, there was a significant effect of

Figure 3. Training induces CA1 dendritic spines shrinkage in young mice and enlargement in old mice. **A**, Representative image of the dorsal hippocampus of *Thy1-GFP(M)* mouse. A white rectangle indicates the area of interest (stratum radiatum of the dorsal CA1 area). Scale bar, 200 μm . **B**, Experimental timeline. Mice were killed immediately after habituation or 2 h of training. **C**, Summarized results show an increase in reward corner preference during training. Data for visits to the sucrose corner is shown. **D**, Representative high-resolution images of dendritic fragments of control and trained young and old *Thy1-GFP(M)* mice. Scale bars, 2 μm . **E**, Summary of results showing a linear dendritic spine density. Data for individual mice are shown (young/control, mice/dendrites = 4/20; young/training, mice/dendrites = 6/38; old/control, mice/dendrites = 4/22; old/training, mice/dendrites = 5/21). **F**, Summarized results show the area of dendritic spines in young and old animals. Graph shows data for individual spines (young/control, mice/spines = 4/1112; young/training, mice/spines = 6/2455; old/control, mice/spines = 4/972; old/training, mice/spines = 5/1158). **G–I**, Frequency distribution of spine areas in young and old, control and trained mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Mean $\pm$ SEM values are shown for **C** and **E**; median $\pm$ IQR values are shown for **F**. Axes are log10 transformed in **G–I** to show differences between the groups.

age on the frequency of perseverative visits ($t = 2.891$, $df = 20$, $p = 0.009$), and only close to significant on perseverative licks ($t = 2.013$, $df = 20$, $p = 0.057$; Fig. 2E). Interestingly, during the mixed-population habituation, the frequency of perseverative visits in old mice was lower than that in the separate populations (67% vs 77%; Student's t test, $t = 2.891$, $df = 20$, $p = 0.009$). The frequency of perseverative licks in young mice was higher in the mixed population compared with separate populations (86 vs 65%; $t = 2.013$, $df = 20$, $p = 0.058$). Finally, we observed a significant effect of training (mixed-effects model, $\chi^2 = 4.645$, $df = 1$, $p = 0.031$; $F_{(1,19)} = 15.68$, $p < 0.001$), but no effect of age ($F_{(1,20)} = 0.641$, $p = 0.433$) or interaction between these factors ($F_{(1,19)} = 0.006$, $p = 0.941$) on the frequency of social visits (Fig. 2F). As confirmed by Sidak's *post hoc* test, there was no significant difference in the frequency of social visits between young and old mice during habituation or training. Both young ($p = 0.036$) and old mice ($p = 0.011$) increased the frequency of social visits during training compared with habituation. Altogether, our data confirm that a high tendency for perseverative visits and social interactions may contribute to the robust place and reward preference in aged mice. Moreover, the frequency of such behaviors is affected by cage mates.

Aging affects training-induced plasticity of dendritic spines

Spatial choice is believed to rely on synaptic plasticity in dCA1 (Bannerman et al., 2012, 2014). Therefore, we analyzed training-induced changes in dendritic spines of young and old mice, as a proxy of training-induced synaptic plasticity (Graziane et al., 2016). Dendritic spines were analyzed after 2 h of training. We chose the 2 h time point for the analysis of dendritic spines as our previous experiments showed significant changes in dendritic spines at this time point after hippocampus-dependent training (Radwanska et al., 2011). Here, we used young (5 months old) and old (18 months old) *Thy1-GFP(M)* mice (Feng et al., 2000) that express GFP in a sparsely distributed population of glutamatergic neurons (Fig. 3A). The animals were trained in separate cages to find sucrose in one cage corner, and they were killed after 2 h of training. Control groups were killed immediately after habituation (Fig. 3B). We observed a significant effect of training (mixed-effects model, $\chi^2 = 0.8299$, $df = 1$, $p = 0.362$; training: $F_{(3,25)} = 52.93$, $p < 0.001$) on the frequency of reward corner visits, indicating that both young and old mice developed a preference for the sucrose corner (Fig. 3C). There was no effect of age ($F_{(1,9)} = 0.9315$, $p = 0.36$) or interaction between age and time ($F_{(3,25)} = 1.601$, $p = 0.214$) on the reward corner preference.

However, the LSD *post hoc* test for planned comparisons found that old mice had a higher preference for the reward corner than young animals at the end of training (2 h; $p < 0.05$), confirming a more robust place preference.

Dendritic spines were analyzed in the stratum radiatum of the dCA1 (Fig. 3A,D), as this region is involved in cognitive processes that require spatial choice (Bannerman et al., 2012, 2014). We observed a significant effect of training (two-way ANOVA, $F_{(1,14)} = 8.126$, $p = 0.013$), but not age ($F_{(1,14)} = 0.0103$, $p = 0.921$) or age and training interaction ($F_{(1,14)} = 1.901$, $p = 0.19$), on dendritic spine density (Fig. 3E). Tukey's multiple comparisons *post hoc* tests revealed that there was no difference in dendritic spine density in any of the groups (young, old, control, or trained). A trend for dendritic spine density to increase after 2 h of training was observed in the old mice, though it was not significant ($p = 0.054$). The analysis of dendritic spines areas showed a significant experimental group effect [Kruskal–Wallis test, $H = 42.21$, $p < 0.001$; Fig. 3F]. *Post hoc* multiple-comparisons Dunn's test confirmed that young control mice had larger spines than the old control mice (0.248 vs $0.214 \mu\text{m}^2$; $p < 0.001$). Moreover, the median spine area in young mice shifted to a smaller value after training (0.248 vs $0.225 \mu\text{m}^2$; $p < 0.001$) and, in contrast, to a larger value in old mice (0.214 vs $0.227 \mu\text{m}^2$; $p < 0.001$). These observations were confirmed when frequency distributions of spines areas were analyzed (Fig. 3G–I). There was an increased frequency of small spines in old mice, compared with young controls (Kolmogorov–Smirnov test, $D = 0.1216$, $p < 0.001$), and in young mice after training, compared with controls ($D = 0.074$, $p < 0.001$; Fig. 3H), while in old mice, the frequency of small spines decreased after training compared with controls ($D = 0.079$, $p = 0.003$; Fig. 3I). Overall, our data show that spatial training in the IntelliCages resulted in shrinkage of dendritic spines in the dCA1 of young mice. In contrast, in old mice, new spines were generated and the median size of spines was increased, suggesting an alternative form of synaptic plasticity. To further validate this hypothesis, we decided to analyze the expression of the postsynaptic protein PSD-95, which controls AMPAR localization at the synapse (Bats et al., 2007; Opazo et al., 2012).

The effect of aging on the correlation between levels of synaptic protein, PSD-95, and dendritic spine size

PSD-95 is a crucial scaffolding protein at mature glutamatergic synapses (Aoki et al., 2001). It promotes the survival of dendritic spines (El-Husseini et al., 2000; Murmu et al., 2013; Meyer et al., 2014) and supports the stability of hippocampus-dependent memories (Fitzgerald et al., 2015). As old mice developed a more robust preference of the reward corner than young animals, we tested whether PSD-95 protein expression is regulated differently in young and old mice during spatial choice training.

To track colocalization of PSD-95 protein with dendritic spines, we performed immunostaining on the brain sections of young and old *Thy1-GFP(M)* mice using specific antibodies (Fig. 4A) to identify PSD-95(+) and PSD-95(−) spines (Fig. 4B). There was no significant effect of age (three-way ANOVA, $F_{(1,29)} = 0.01$, $p = 0.92$) or training ($F_{(1,29)} = 0.728$, $p = 0.401$), but PSD-95 expression ($F_{(1,29)} = 63.32$, $p < 0.001$) significantly affected dendritic spines density (Fig. 4C). Moreover, there was a significant interaction among training, age, and PSD-95 ($F_{(1,29)} = 5.912$, $p = 0.021$). LSD *post hoc* tests (for planned comparisons only) confirmed that there were more PSD-95(−) spines in the

old control ($p = 0.001$) and trained ($p < 0.001$) mice, compared with young control and trained mice, respectively. Training did not affect the density of PSD-95(+) and PSD-95(−) spines in young mice, but significantly increased PSD-95(−) spine density in old mice ($p = 0.01$; compared with the old controls). Old trained mice had more PSD-95(−) than PSD-95(+) spines ($p = 0.001$), and old mice overall had fewer PSD-95(+) spines than young mice.

Next, we determined the levels of PSD-95 protein per dendritic spine. There was a significant experimental group effect on the area of PSD-95 puncta (Kruskal–Wallis test, $H = 75.64$, $p < 0.001$; Fig. 4D). PSD-95 puncta in young control mice were larger, compared with the old control mice (0.027 vs $0.013 \mu\text{m}^2$; Dunn's *post hoc* tests, $p < 0.001$), further indicating a downregulation of PSD-95 expression in aged animals. Moreover, the median area of PSD-95 puncta was smaller in the trained animals, compared with the age-paired control groups (young: 0.027 vs $0.015 \mu\text{m}^2$, $p < 0.001$; old: 0.013 vs $0.010 \mu\text{m}^2$, $p < 0.01$), indicating that the training downregulated PSD-95 expression.

Training-induced enlargement of dendritic spines and shrinkage of PSD-95 puncta in old mice suggest decorrelation of these parameters. To test whether such change also occurs at the level of individual spines, we compared the correlation strength of PSD-95 area and spine area (Spearman correlation, r ; Fig. 4E–G). The parameters strongly correlated in all experimental groups (Spearman correlation, $p < 0.001$ for all groups), indicating that large dendritic spines have higher expression of PSD-95 protein than small spines. Furthermore, the regression lines describing the correlations in young and old control groups showed a significant slope difference (ANCOVA, $F_{(1,822)} = 21.82$, $p < 0.001$), indicating that dendritic spines in young mice had larger PSD-95 areas compared with those of the same size in old mice (Fig. 4E). This difference was size dependent and most pronounced for large dendritic spines. A similar shift of the regression lines was observed in young mice after training, compared with young control mice (slopes: $F_{(1,1517)} = 26.30$, $p < 0.001$; Fig. 4F). There was no difference in the slopes of the regression lines of old mice after training compared with the old control mice ($F_{(1,536)} = 2.783$, $p = 0.096$; Fig. 4G). The drop in elevation of the regression line after training in the old mice ($F_{(1,537)} = 7.385$, $p = 0.007$), indicate that dendritic spines of the same size had smaller PSD-95 puncta, but this shift did not depend on the size of the dendritic spine. Moreover, we observed a significant effect of age (two-way ANOVA, $F_{(1,13)} = 15.38$, $p = 0.002$) and training ($F_{(1,13)} = 5.983$, $p = 0.029$), but no interaction between these factors ($F_{(1,13)} = 0.009$, $p = 0.925$) on the strength of PSD-95 and dendritic spine area correlation (Spearman correlation, r ; Fig. 4H). The LSD *post hoc* tests for planned comparisons confirmed that the correlation of PSD-95 area and dendritic spines area was weaker for both old control ($p = 0.016$) and trained ($p = 0.016$) mice, compared with young mice (Fig. 4H). Furthermore, coefficient of variation (CV) analysis of PSD-95 and dendritic spines areas found a significant effect of age (two-way ANOVA, $F_{(1,15)} = 9.275$, $p = 0.008$), a minor effect of training ($F_{(1,15)} = 4.374$, $p = 0.054$), and no interaction between these factors ($F_{(1,15)} = 0.546$, $p = 0.471$; Fig. 4I). The LSD *post hoc* tests for planned comparisons found significantly higher CV in old trained mice compared with young trained mice ($p = 0.011$), indicating a higher variability of PSD-95 areas in old animals. Overall, our data indicate that training in young mice induced dendritic spine shrinkage accompanied by downregulation of PSD-95 expression. Training in old mice induced dendritic spines enlargement and PSD-95 puncta shrinkage, presumably, decorrelating these parameters.

Figure 4. Training downregulates PSD-95 expression in young and old mice and decreases the correlation between areas of PSD-95 puncta and dendritic spines in old mice. **A**, Representative images of PSD-95 immunostaining colocalized with dendritic fragments of *Thy1-GFP(M)* mice. Scale bars, 2 μm. **B**, Examples of dendritic spines: PSD-95(+) and PSD-95(-). **C**, Summary of results showing linear density of spines with and without PSD-95. Graph shows data for animals (young/control, mice/dendrites = 4/20; young/training, mice/dendrites = 6/38; old/control, mice/dendrites = 4/22; old/training, mice/dendrites = 4/21). **D**, Summarized results showing area of PSD-95 puncta. Graph shows data for total PSD-95 area per individual spines (young/control, mice/spines = 4/497; young/learning = 6/1024; old/control = 4/329; old/learning = 4/214). **E–G**, Correlation of PSD-95 area and dendritic spine area in young and old, control and trained mice. **H**, Calculation of Spearman's rank correlation coefficient of dendritic spine area and PSD-95 area (young/control, $n = 4$; young/training, $n = 5$; old/control, $n = 4$; old/training, $n = 4$). **I**, Coefficient of variation determined as a ratio between size of PSD-95 and spine areas. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Mean $\pm$ SEM values are shown for **C**, **H**, and **I**; median $\pm$ IQR values are shown for **D**. Linear regression lines together with their 95% confidence bands are shown for **E–G**.

Spatial choice and nonspatial training induce different patterns of synaptic plasticity

We next studied whether synaptic changes observed during training in young mice were related to spatial or nonspatial aspects of training. To this end, young adult mice were trained in nonspatial sucrose training (sucrose in both active corners), or spatial aversive training (quinine in one corner, water in the other). The control mice were killed after habituation (Fig. 5A). There was a significant effect of training on visits and licks (mixed-effects model; visits: $F_{(1,6)} = 9.139$, $p = 0.023$; licks: $F_{(1,6)} = 9.356$, $p = 0.022$), but no effect of time (visits: $F_{(1,363,8,180)} = 3.325$, $p = 0.098$; licks: $F_{(1,294,6,037)} = 1.167$, $p = 0.342$) or interaction

between these factors (visits: $F_{(3,18)} = 1.45$, $p = 0.262$; licks: $F_{(3,14)} = 3.085$, $p = 0.062$) on corner preference (Fig. 5B.i,B.ii). Mice in the sucrose group did not show spatial preference after 2 h of training (LSD *post hoc* tests for planned comparisons, $p > 0.05$ for visits and licks at 2 h vs H). In contrast, mice in the quinine group significantly decreased the preference to the quinine corner ($p < 0.05$ for visits and licks at 2 h vs H), indicating the formation of spatial and nonspatial aversive memories.

The analysis of dendritic spines showed that there was a significant effect of training on spine density in the three experimental groups (one-way ANOVA, $F_{(2,9)} = 2.343$, $p < 0.001$; Fig. 5C.i,C.ii). Spine density was increased in the sucrose group, compared with

Figure 5. In young mice, aversive spatial choice training induces dendritic spines shrinkage, while appetitive nonspatial training results in dendritic spines enlargement. **A**, Experimental timeline and IntelliCage setups during nonspatial reward (sucrose in both corners) or spatial aversive training (quinine in one corner). The control group (water in both corners) was killed after habituation. **B**, Summary of data showing that mice avoided the corner with quinine, measured as visits (**B.i**) and licks (**B.ii**), and did not change corner preference when sucrose was in both corners. **C**, Analysis of dendritic spines. **C.i**, Representative microphotographs of dendritic fragments of the young *Thy1-GFP(M)* mice drinking water (control group), quinine or sucrose. Scale

the control group ($p = 0.015$), and there was no significant difference between the quinine and control groups ($p > 0.05$). We also observed a decrease in the median spine area in the quinine group compared with the control group (0.198 vs $0.207 \mu\text{m}^2$), while an increase in the sucrose group (0.207 vs $0.213 \mu\text{m}^2$; Kruskal–Wallis test with Dunn's *post hoc* tests, $H = 15.03$, $p < 0.001$; Fig. 5C.iii).

Furthermore, an increased frequency of PSD-95(+) spines was found in the sucrose group compared with the controls ($p < 0.05$), and no change in spine frequency in the quinine group (two-way RM ANOVA with Tukey's multiple comparisons test; effect of training: $F_{(2,9)} = 15.27$, $p = 0.001$; PSD-95: $F_{(1,9)} = 333.7$, $p < 0.001$; interaction: $F_{(2,9)} = 3.114$, $p = 0.094$; Fig. 5D.i,D.ii). The quinine group also had decreased PSD-95 puncta area per dendritic spine ($0.041 \mu\text{m}^2$) compared with the control group ($0.050 \mu\text{m}^2$, $p < 0.001$), while the sucrose group had increased PSD-95 puncta area ($0.060 \mu\text{m}^2$; $p < 0.001$; Fig. 5D.iii; Kruskal–Wallis test with Dunn's *post hoc* tests, $H = 50.89$, $p = 0.001$).

There was a strong correlation between the PSD-95 area and the dendritic spine area in all experimental groups (Spearman correlation, $p < 0.001$ for all groups; Fig. 5E). The regression line describing the correlation in the quinine group was shifted downward and differed in slope, compared with the controls (ANCOVA, $F_{(1,2752)} = 11.53$, $p < 0.001$), indicating that the quinine group spines had relatively smaller PSDs than the control group, and this difference was most pronounced in large spines. In the sucrose group, the regression line was shifted upward, compared with the control group, and differed in elevation (ANCOVA, $F_{(1,2924)} = 7.345$, $p = 0.007$; Fig. 5E), indicating that the PSDs were enlarged. We did not observe any effect of training on the correlation strength (one-way ANOVA, $F_{(2,9)} = 0.704$, $p = 0.52$; Fig. 5F) or CV (one-way ANOVA, $F_{(2,9)} = 1.118$, $p = 0.368$; Fig. 5G) of PSD-95 puncta and dendritic spine area. Overall, our experiments show that the pattern of synaptic changes observed in spatial training groups (sucrose and quinine) was similar and was characterized by the downregulation of synaptic PSD-95 protein and shrinkage of dendritic spines. In nonspatial sucrose training, dendritic spines were enlarged, and the expression of PSD-95 increased. In all experimental groups, the changes in the areas of dendritic spines and PSD-95 correlated. Thus, our data suggest the role of PSD-95 protein in the dCA1 for both spatial choice and nonspatial memory in young mice.

Role of dCA1 PSD-95 in spatial choice of young and old mice

To test the function of PSD-95 protein in dCA1 in spatial choice we used LVs encoding shRNA targeted to PSD-95 mRNA (LV: H1-shRNA_PSD-95-Ub-GFP; shPSD95; Schlüter et al., 2006).

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bars, $2 \mu\text{m}$. **C.ii, C.iii**, Summary of data showing spine density (mean values for animals; control, mice/dendrites = $4/31$; quinine = $4/28$; sucrose = $4/27$; **C.ii**) and dendritic spines area (individual spine data; control, mice/spines = $4/2105$; quinine, mice/spines = $4/1653$; sucrose, mice/spines = $4/1171$; **C.iii**). **D**, Analysis of dendritic spines with PSD-95 puncta. **D.i**, Representative microphotographs of dendritic fragments [Thy1-GFP(M)] with PSD-95 immunostaining (scale bars, $2 \mu\text{m}$). **D.ii**, Summary of data showing the density of PSD-95(+) and PSD-95(−) spines (individual mice data; control, mice/dendrites = $4/31$; quinine, mice/dendrites = $4/28$; sucrose, mice/dendrites = $4/27$). **D.iii**, Area of PSD-95 puncta (individual spine data; control, mice/spines = $4/2105$; quinine = $4/1653$; sucrose = $4/1171$). **E**, Correlation of PSD-95 and dendritic spine areas in control, quinine, and sucrose mice. Linear regression lines, together with their 95% confidence bands are shown. **F**, Calculation of Spearman's rank correlation coefficient of the dendritic spine area and PSD-95 area. **G**, Coefficient of variation calculated for individual mice (control, $n = 4$; quinine, $n = 4$; sucrose, $n = 4$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Mean $\pm$ SEM values are shown for **B.i,ii**, **C.ii**, **D.ii**, **F**, and **G**; median $\pm$ IQR values are shown for **C.iii** and **D.iii**.

For control, a lentivirus coding shRNA designed for Renilla luciferase (LV:H1-shRNA_luciferase; shLuc) was used. Young (5 months old) and old (20 months old) C57BL/6J mice had LVs, shPSD95 or shLuc, stereotactically injected into dCA1 before the training in the IntelliCages (Fig. 6A). The post-training analysis of the hippocampal sections confirmed that shPSD95 expression was limited to dCA1 (bregma > -2.5 mm; Fig. 6B,C), and it effectively downregulated PSD-95 protein levels in young mice (Student's *t* test, $t = 4.243$, $df = 6$, $p = 0.005$) and old mice ($t = 2.292$, $df = 9$, $p = 0.048$; Fig. 6D). Importantly, shPSD95 did not impair basal mouse activity and liquid consumption (Fig. 6E, F), indicating no gross behavioral impairments.

The activity of young mice with shLuc and shPSD95 (Fig. 6E) was significantly affected by the training (two-way RM ANOVA, visits: $F_{(1,16)} = 15.59$, $p = 0.001$; licks: $F_{(1,16)} = 31.6$, $p < 0.001$), but not by shRNA expression (visits: $F_{(1,16)} = 0.099$, $p = 0.757$; licks: $F_{(1,16)} = 0.022$, $p = 0.883$). There was also no significant interaction between training and shRNA (visits: $F_{(1,16)} = 1.211$, $p = 0.287$; licks: $F_{(1,16)} = 0.505$, $p = 0.488$). The Sidak's *post hoc* tests confirmed that training increased the frequency of visits and licks in the shLuc group ($p < 0.01$). However, in the shPSD95 group, training increased the daily number of licks ($p < 0.01$), but not visits ($p > 0.05$). Thus, the depletion of PSD-95 prevented a training-induced rise in the activity of young mice.

During training, there was also a significant effect of time on a sucrose corner preference of young mice (mixed-effects model for visits: $\chi^2 = 14.30$, $df = 1$, $p < 0.001$; time: $F_{(5,77)} = 15.59$, $p < 0.001$; licks frequency: $\chi^2 = 31.62$, $df = 1$, $p < 0.001$; time: $F_{(3,031,46,68)} = 40.37$, $p < 0.001$; Fig. 6F). This indicates that young mice in both experimental groups formed spatial and nonspatial memory. However, we also noticed a significant effect of shRNA targeting PSD-95 ($F_{(1,16)} = 12.13$, $p = 0.003$) and the interaction of time and shRNA ($F_{(5,77)} = 5.82$, $p < 0.001$) on the change of visit preference. Mice with depleted PSD-95 levels showed a lower preference for the sucrose corner during the initial phase of the training than the controls ($p < 0.001$, for 1 and 2 h; Fig. 6F.i), indicating impairment of spatial choice. The licks preference analysis revealed that there was no significant effect of silencing PSD-95 gene expression ($F_{(1,16)} = 0.118$, $p = 0.736$) or interaction of shRNA and time ($F_{(5,77)} = 0.989$, $p = 0.43$), indicating that nonspatial memory was preserved in young mice despite the downregulation of PSD-95 (Fig. 6F.ii).

Since corner patrolling, perseverative visits, and social interaction may affect mice performance in spatial choice test, we analyzed the effect of shPSD95 on these behaviors. The analysis of the patrolling visits revealed a significant effect of training (two-way RM ANOVA, $F_{(1,11)} = 21.68$, $p < 0.001$), but no effect of shPSD95 ($F_{(1,11)} = 0.75$, $p = 0.405$) or interaction of shRNA and training ($F_{(1,11)} = 3.156$, $p = 0.103$), on their frequency (Fig. 6G). The Sidak's *post hoc* tests showed, however, that the training decreased the frequency of patrolling visits only in the shLuc group ($p < 0.01$), but not in the shPSD95 group ($p > 0.05$). Thus, the depletion of PSD-95 prevented a training-induced decrease in cage patrolling. There was no effect of shRNA on the frequency of perseverative visits (Student's *t* test, $t = 0.956$, $df = 11$, $p = 0.36$) and licks ($t = 0.737$, $df = 11$, $p = 0.477$) during habituation (Fig. 6H.i,H.ii). Finally, there was no effect of shRNA ($F_{(1,11)} = 0.199$, $p = 0.664$), but there was a significant effect of training (two-way RM ANOVA, $F_{(1,11)} = 57.77$, $p < 0.001$), on the frequency of social visits (Fig. 6I). As confirmed by the *post hoc* tests, the frequency of social visits was increased during training in both experimental groups ($p < 0.001$ for both). Thus,

Figure 6. PSD-95 protein in dCA1 is required for spatial choice in young mice, but not old mice. **A**, Experimental timeline. After the injection of shPSD95 or shLuc into dCA1, mice were trained in the IntelliCage. First, the animals were habituated to the cage, with water in both corners. Next, during spatial training, a bottle with sucrose was placed in one cage corner. **B**, Representative confocal scan of the dorsal hippocampus with local overexpression of shRNA targeted to PSD-95. Scale bar, 100 μ m. **C**, The map of shPSD95 transfection. Maximal and

unlike the young mice that were trained separately (Fig. 1), the young mice that were trained together expressing shLuc and shPSD95 increased the frequency of social interactions during training. This observation confirms that social context (old mice or mice with depleted PSD-95 in dCA1) affects the social behavior of young animals.

Similarly to the young animals, activity of aged mice with shLuc and shPSD95 was significantly affected by the training (two-way RM ANOVA, effect of time; visits: $F_{(1,13)} = 7.844$, $p = 0.015$; licks: $F_{(1,12)} = 27.10$, $p < 0.001$; Fig. 6*f*), but there was no effect of shRNA (visits: $F_{(1,13)} = 1.553$, $p = 0.235$; licks: $F_{(1,12)} = 2.457$, $p = 0.143$) or interaction between time and shRNA (visits: $F_{(1,13)} = 1.946$, $p = 0.184$; licks: $F_{(1,12)} = 0.669$, $p = 0.429$). The Sidak's *post hoc* tests indicated that, while in shLuc group both visits and licks were more frequent during training compared with the habituation ($p < 0.01$), in the shPSD95 group training increased only the number of licks, but not the number of visits (licks: $p < 0.01$; visits: $p > 0.05$). Thus, depletion of PSD-95 prevented a training-induced rise in the activity of aged mice.

Moreover, we found a significant effect of time ($F_{(5,55)} = 52.59$, $p < 0.001$), but no effect of shRNA ($F_{(1,12)} = 0.77$, $p = 0.40$) or interaction between shRNA and time ($F_{(5,55)} = 1.912$, $p = 0.107$), on visit preference in old mice (Fig. 6*K.i*). We also observed a significant effect of time (mixed-effects model, $\chi^2 = 21.56$, $df = 1$, $p < 0.001$; training: $F_{(1,977,21.35)} = 137.4$, $p < 0.001$), but no effect of shRNA ($F_{(1,12)} = 2.93$, $p = 0.113$) or interaction of shRNA and time ($F_{(5,54)} = 1.362$, $p = 0.253$), on licks preference (Fig. 6*K.ii*). The absence of significant differences in reward corner preference during training between shLuc and shPSD95 groups was also confirmed by Sidak's *post hoc* tests. Thus, the depletion of PSD-95 levels affected neither spatial choice nor nonspatial memory in old mice.

The analysis of the patrolling visits revealed that there also was no effect of time ($F_{(1,12)} = 2.608$, $p = 0.132$) or shRNA ($F_{(1,12)} = 0.457$, $p = 0.512$), but a significant time and shRNA interaction on their frequency ($F_{(1,12)} = 7.427$, $p = 0.018$; Fig. 6*L*). The Sidak's *post hoc* tests confirmed that the frequency of the patrolling visits was decreased during training in the shLuc group, but not in the shPSD95, indicating that the depletion of PSD-95 impaired training-induced decrease of cage patrolling. No significant difference was observed between the shPSD95 and shLuc groups in the frequency of perseverative visits (Student's *t* test, $t = 1.727$, $df = 13$, $p = 0.108$) and licks ($t = 2.036$, $df = 13$, $p = 0.063$; Fig. 6*M.i,M.ii*). Finally, we observed a significant effect of

training on the frequency of social visits both in the shLuc group (Wilcoxon matched-pairs signed-rank test, $p = 0.031$) and the shPSD95 group ($p = 0.031$; Fig. 6*N*). Overall, our data indicate that dCA1-targeted downregulation of PSD-95 impaired spatial choice in young mice, but it had no significant effect on the spatial choices of aged animals. Interestingly, the depletion of PSD-95 also prevented a training-induced increase of mouse activity and a decrease of the patrolling visits independent of the age of the mice. However, since these parameters did not differ between the experimental groups during training (shLuc vs shPSD95), the impairments could not explain poor spatial choices in the young shPSD95 group.

Discussion

Accumulating data indicate that synaptic plasticity in dCA1 is essential for spatial choice, rather than spatial memory (Bannerman et al., 2012, 2014). Here, mice living in a group were trained to make spatial choices between two visually identical cage corners. We demonstrate that aversive and appetitive spatial choice training in young mice induced similar synaptic alterations in the dCA1 that were characterized by dendritic spine shrinkage and the downregulation of PSD-95 protein levels. Moreover, spatial choice in young mice is impaired by local downregulation of PSD-95 in the dCA1. Thus, our data support the role of PSD-95-dependent synaptic plasticity in dCA1 in spatial choice of young animals. Surprisingly, dCA1-targeted depletion of PSD-95 does not affect spatial choice in aged mice, suggesting that neuronal bases of spatial choice change with aging.

Old mice are impaired in hippocampus-dependent tasks, including spatial choice in the radial maze or Barnes maze (Ammassari-Teule et al., 1994; Bach et al., 1999). However, old animals can use alternative nonspatial behavioral strategies to solve tasks that require the hippocampus. For example, young mice use spatial strategy in the Barnes maze, while old animals tend to use a nonspatial serial search strategy (Bach et al., 1999). Similarly, in our study old mice used alternative behavioral strategies to find a reward corner in the IntelliCage. They relied on social observations and perseverative behavior (visiting and drinking only from one corner even during habituation), strategies commonly used also by elderly people (Ridderinkhof et al., 2002). Previously, it was shown that social interactions improved place learning of old APP (amyloid precursor protein) mutant mice living in a group (Kiryk et al., 2011). Here, we demonstrate for the first time that both healthy old and young mice use social information during spatial choice training, and, accordingly, mouse behavior depends on social context (e.g., it is altered in the company of mice that use an alternative strategy because of their age or the functional impairments of dCA1). Interestingly, perseverative behavior is observed also in animals with hippocampal lesions and mutations (Brookes et al., 1983; Hoeffer et al., 2008). The animals with impaired hippocampi tend to choose strategies that relay on other brain regions (e.g., striatum; Brookes et al., 1983; Hoeffer et al., 2008). In agreement with this hypothesis, local depletion of PSD-95 protein in the CA1 area of aged mice did not affect place preference, suggesting that aged mice use a dCA1-independent strategy during spatial choice task. It is, however, possible that spatial choice in old mice involves dCA1, but not PSD-95.

Interestingly, except for the effect of PSD-95 depletion in dCA1 on spatial choice in young mice, this local molecular manipulation prevented a training-induced rise in mouse activity

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minimal transfections are presented. **D.i**, Representative microphotographs of PSD-95 immunostaining. Scale bars, 5 μ m. **D.ii**, Summary of data showing that the local expression of shPSD95 downregulated PSD-95 expression in the CA1 area of young mice. **D.iii**, Summary of data showing that shRNA for PSD-95 downregulated PSD-95 levels in the CA1 of old mice. **E**, Summary of data showing the activity of young mice during habituation and training. Daily visits (**E.i**) and licks of all liquids (**E.ii**) are shown. **F**, Summarized data showing that downregulation of PSD-95 expression in the CA1 area decreased place preference in young animals (**F.i**), but did not affect sucrose preference (**F.ii**). **G–I**, Summary of frequency data of patrolling visits (without licks; **G**), perseverative visits (**H.i**) and licks (**H.ii**; to preferred corner), and social visits (following another mouse to the corner with an intervisit interval of < 1 s; **I**) during habituation and training. **J**, Summary of data showing the activity of old mice during habituation and training. Daily visits (**J.i**) and licks of all liquids (**J.ii**) are shown. **K**, Summarized data for old mice, showing the effect of local downregulation of PSD-95 expression in the CA1 area by shRNA on place preference (**K.i**) and sucrose preference (**K.ii**). **L–N**, Summary of data showing the frequency of patrolling visits (without licks; **L**), perseverative visits (**M.i**) and licks (to preferred corner; **M.ii**), and social visits during habituation and training (**N**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Mean $\pm$ SEM values are shown on graphs for **D–M**; median $\pm$ IQR values are shown for **N**.

and a decrease in the frequency of patrolling visits, both in young and old mice. These effects may result from the interaction between dCA1 and nucleus accumbens (NAc), an interface between the limbic and the motor systems. As recently shown, NAc integrates dCA1 information about reward-predicting environment that can be used to guide appetitive behavior and arousal (Trouche et al., 2019). Thus, some functions of dCA1 that require PSD-95 seem to be preserved in old mice.

Most studies to date link cognitive processes with LTP of synaptic transmission (Bliss and Collingridge, 1993; Morris et al., 2003), reflected on a structural level as growth and the addition of new dendritic spines (harboring glutamatergic synapses; Restivo et al., 2009; Radwanska et al., 2011; Mahmoud et al., 2015; Aziz et al., 2019), long-lasting growth of PSDs (excitatory synapses; Śliwińska et al., 2020), and recruitment of AMPA receptors into existing spines (Matsuo et al., 2008). However, accumulating data indicate that spatial choice may also rely on the LTD of synaptic transmission (Kemp and Manahan-Vaughan, 2007; Connor and Wang, 2016) that is characterized by the shrinkage of dendritic spines and PSDs or spine elimination (Nägerl et al., 2004; Zhou et al., 2004). Although structural correlates of learning-induced LTD has been rarely documented *in vivo*, spatial choice in the Barnes maze has been found to induce LTD in the CA1 area and interventions that prevent LTD, disrupted formation, and updating of spatial choices (Kemp and Manahan-Vaughan, 2004; Griffiths et al., 2008; Goh and Manahan-Vaughan, 2013).

Here, we demonstrate that spatial choice training in young mice induced dendritic spine shrinkage in the stratum radiatum of dCA1. Moreover, the size of PSD-95 clusters in spines was also decreased, indicating downregulation of PSD-95 expression. A similar pattern of synaptic changes was observed after aversive spatial training, while nonspatial reward training resulted in dendritic spine enlargement and an increased area of PSD-95 puncta. Since PSD-95 protein controls the localization of AMPARs at the synapse (Bats et al., 2007; Opazo et al., 2012) and AMPAR currents (Béique et al., 2006; Schlüter et al., 2006; Opazo et al., 2012), then these morphologic and molecular changes observed in mice that underwent spatial training point to at least temporal weakening of synapses in the CA1 circuit. Still, it has to be established whether spatial choice training in the close-to-ethologic condition induces LTD in dCA1, in a way that was similar to spatial choice in the Barnes maze (Kemp and Manahan-Vaughan, 2004; Griffiths et al., 2008; Goh and Manahan-Vaughan, 2013). In support of this assumption, shPSD95, which impaired spatial choice of young mice in our experiments, not only downregulates PSD-95 levels and prevents maturation of dendritic spines but also occludes LTD (Ehrlich et al., 2007; Xu et al., 2008). The shrinkage of dCA1 synapses during spatial choice may reflect the role of the hippocampus in behavioral inhibition to prevent wrong choices (Bannerman et al., 2012, 2014). However, understanding how synaptic shrinkage enables the selection of the rewarded corner requires further studies.

Training-induced changes in dendritic spines further support an alternative neuronal strategy for spatial choice in aged mice. In old mice, spatial choice training increased the density and size of dendritic spines, while the expression of PSD-95 per spine decreased. Thus, the pattern of synaptic changes did not resemble the synaptic weakening observed during spatial choice training in the young mice. Instead, it bears components of nonspatial reward training (increased density and growth of dendritic spines) as well as spatial training (downregulation

of PSD-95). Moreover, we observed that old mice have smaller dendritic spines, fewer spines with PSD-95, decreased size of PSD-95 clusters per spine, and decorrelation of PSD-95 levels with dendritic spine size, compared with young mice. The shrinkage of dendritic spines and decreased levels of synaptic proteins are in agreement with earlier studies in aged animals (Ganeshina et al., 2004; Counts et al., 2014; Rogers et al., 2017; Śliwińska et al., 2020). Since small dendritic spines with low levels of PSD-95 are less stable than large spines (Cane et al., 2014), our observation also supports the notion that dendritic spines in aged mice are less stable (Mostany et al., 2013). Ours is the first study to show the decorrelation of PSD-95 expression and dendritic spine size in old animals. This phenomenon seems to be specific for the aged brain as the correlation between these two parameters was not affected by spatial choice or nonspatial reward training in young mice. Previously, the uncoupling of PSD and spine volumes was observed after glutamate uncaging in cell culture. However, it was transient (<7 min) and presumably caused by a slower PSD growth compared with the spine (Bosch et al., 2014; Meyer et al., 2014). Moreover, NMDAR-dependent LTP in organotypic hippocampal slices was shown to increase the correlation between PSD size and dendritic spine volume (Borczyk et al., 2019). Precise reasons for dendritic spine volume and PSD-95 expression correlation remain unknown. However, since PSD-95 anchors AMPA and NMDA receptors (Sheng and Kim, 2011; Chen et al., 2015), PSD-95 levels may affect Ca^{2+} signaling. Computational models predict that Ca^{2+} influx and spine size both crucially determine long-term synaptic stability, synaptic strength, and the distribution of dendritic spine sizes (O'Donnell et al., 2011). Accordingly, all these processes may be altered with age, resulting in poor specificity and stability of cognitive maps in the dCA1 of old animals (Barnes et al., 1997; Attardo et al., 2018) and switch to a PSD-95-independent strategy during training.

Overall, our data show that spatial choice in young animals involves PSD-95-dependent processes in the dCA1. Surprisingly in aged animals, although PSD-95 in the dCA1 is depleted, spatial choice remains intact. To obtain reward in a complex environment, old mice use an alternative behavioral strategy that relies on social observation and perseverative visits to the reward corner.

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