Genetic ablation of dentate hilar somatostatin-positive GABAergic interneurons is sufficient to induce cognitive impairment

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Abstract

Aging is often associated with a decline in cognitive function. A reduction in the number of somatostatin-positive (SOM+) interneurons in the DG has been described in cognitively impaired but not in unimpaired aged rodents. However, it remains unclear whether the reduction in SOM+ interneurons in the DG hilus is causal for age-related cognitive dysfunction. We hypothesized that hilar (SOM+) interneurons play an essential role in maintaining cognitive function and that a reduction in the number of hilar (SOM+) interneurons might be sufficient to induce cognitive dysfunction. Hilar (SOM+) interneurons were ablated by expressing a diptheria toxin transgene specifically in these interneurons. An AAV-EF1α-mCherry-flex-dtA construct was stereotaxically injected (bilaterally) into the DG hilus of young adult Sst-IRES-Cre mice, which resulted in an approximately 50% reduction in the number of SOM+ neurons and also a reduction in the number of GAD-67+ neurons in the DG hilus. c-Fos staining was increased in DG and CA3 but not in CA1. Behavioral testing, which started 21 days after the stereotaxic injections, revealed a reduced recognition index in the novel object recognition test, a reduction in the percentage of correct alternations in the Y maze tests, and increased latencies and path lengths in the learning and the reversal learning phase of the Morris water maze. Our results show that partial genetic ablation of SOM+ hilar interneurons is sufficient to increase activity in DG and CA3, as has been described to occur with aging, and to induce an impairment of learning and memory functions. Thus, partial ablation of hilar SOM+ interneurons may be a significant contributing factor to age-related cognitive dysfunction. These mice may also be useful as a cellularly defined model of hippocampal aging.

Keywords: **Cognitive impairment; GABAergic interneurons; Aging and Diphtheria toxin**

Introduction

Normal cognitive aging is characterized by accelerated declines in memory from early adulthood (Salthouse, 2019), and the factors behind this process are not entirely clear. Increasing age is a risk factor for postoperative cognitive deficits, which were previously called postoperative cognitive dysfunction (POCD) (Berger et al., 2015, 2018) and are now referred to as postoperative neurocognitive disorder (poNCD)(Evered et al., 2018). Postoperative cognitive deficits can persist for many months after surgery, e.g., in patients > 60 years of age, approximately 10% of patients displayed cognitive dysfunction three months after surgery, compared to 3% in a control group(Moller et al., 1998). Click or tap here to enter text.The molecular and cellular mechanisms underlying cognitive impairments in older individuals remain poorly defined. Mounting evidence points to changes in GABAergic function as a major causal factorClick or tap here to enter text.. Indeed, GABA levels in frontal and in parietal areas decrease with age(Gao et al., 2013), and GABA levels are substantially reduced in hippocampus, striatum and cortex in aged mice (18 months)(Duarte et al., 2014). Such declines in GABA levels may suggest that functional deficits in the GABA system contribute to age-related cognitive dysfunction. Moreover, in human postmortem studies it has been found that the expression of the $GABA_A$ receptor α5 subunit, which has previously been shown to play an important role in modulating learning and memory functions in the hippocampus(Engin et al., 2015, 2020), is decreased in aged human individuals(Lin et al., 2021). Decreased GABA neurotransmitter levels were also observed in the CSF of healthy humans with aging(Bareggi, 1982).

One of the most studied brain structures affected by aging is the hippocampus, which plays an important role in learning and in the formation and consolidation of memory(Bartsch & Wulff, 2015). During aging, the hippocampus shows a decrease in volume, which correlates with a decline in learning and memory(Driscoll et al., 2006). Importantly, GABAergic interneurons have been linked to memory processes. Optogenetic inhibition of hilar GABAergic interneuron activity impairs spatial memory (Andrews-Zwilling et al., 2012). An immunohistochemical study analyzing interneurons in different hippocampal subregions revealed that in aged memory-impaired rats the number of SOM+ interneurons in the DG hilus is significantly smaller than in age-unimpaired rats(Spiegel et al., 2013). Recent studies in humans and rodents have shown that

the different hippocampal subregions are differentially affected by aging. In human subjects, older individuals have increased activity in CA3/DG during while performing pattern separation tasks(Yassa et al., 2011), and in rodents, aging-related dysfunction is associated with hyperexcitability of CA3 pyramidal cells but hypo-excitability of CA1 pyramidal cells(Oh et al., 2016). Causal relationships between dysfunction in DG, CA3, and CA1 are unclear, but it has been speculated that hypo-excitability of CA1 pyramidal cells may occur in compensation for DG/CA3 hyperactivity, as the DG/CA3 hyperactivity precedes changes in CA1(Yassa et al., 2011). Furthermore, Koh et al. (2013) reported that selective positive allosteric modulation of α5$GABA_A$ receptors improves cognitive function in aged rats with memory impairment(Koh et al., 2013).
It has been shown that α5-$GABA_A$ receptor-mediated tonic inhibition in the DG plays an important role in ensuring normal cognitive functioning under high-interference memory conditions (Engin et al., 2015). Recent evidence supports the cognitive salience of inhibitory interneurons and their role in maintaining balanced excitation-inhibition in the aging human brain(Jessen et al., 2017; Yassa et al., 2011), and studies have shown interneurons to be specifically vulnerable in brain aging (Spiegel et al., 2013; Thomé et al., 2016). Fast spiking interneuron deficits and secondary alterations in high frequency gamma cortical network oscillations were noted in aged mice, the latter being fundamental to higher order information processing (Jessen et al., 2017; Sohal et al., 2009). Reductions of SOM-immunoreactive interneurons were observed in the temporal cortex in AD patients(Waller et al., 2020). In addition, age-related cognitive deficits have been found to be correlated with a decrease in SOM-positive interneurons in the DG hilus in rodents(Koh et al., 2014; Spiegel et al., 2013). However, it remains unclear whether the reduction in SOM+ interneurons in the DG hilus is directly related to or even causal for age-related cognitive dysfunction.

To understand why elderly patients are more prone to poNCD than younger patients, it will be helpful to better understand the mechanisms that contribute to decreased cognitive function in the elderly. Therefore, we studied the role of reduced function and number of somatostatin-positive interneurons for age-related cognitive deficits. We hypothesized that hilar (SOM+) interneurons play an essential role in maintaining cognitive function and that a reduction in the number of hilar (SOM+) interneurons might be sufficient to induce cognitive dysfunction. To test our hypothesis that loss of numbers of these interneurons underlies age related cognitive dysfunction at least in

part, we ablated SOM+ interneurons in the DG hilus. The animals created in this process will serve as a cellularly defined mouse model of age-related cognitive dysfunction and will be useful for testing novel preventative or therapeutic strategies.

Materials and Methods

Mice

Adult Sst-IRES-Cre transgenic mice (Stock no. 013044, The Jackson Laboratory) were crossed with C57BL/6J mice (Stock no. 000664, The Jackson Laboratory). Hemizygous adult mice of both sexes (3-4 months) were used for all studies. All mice were housed in a climate-controlled room (temperature: 22 ± 2°C; humidity: 55 ± 5%) and maintained on a 12:12 light-dark cycle with food and water ad libitum. The maintenance and handling of mice were consistent with the Guide for the Care and Use of Laboratory Animals of the National Research Council. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois Urbana-Champaign.

Stereotactic surgery

To generate mice with a partial ablation of the SOM+ interneurons in the dentate hilus, AAV5-EF1α-mCherry-flex-dtA (construct-387-aav2-5 from the viral vector core of the Canadian Neurophotonics Platform), was injected bilaterally the DG hilus of Sst-IRES-Cre mice (3-4 months of age). Mice received ketoprofen 5mg/kg, s.c., and atropine 0.04 mg/kg, s.c. before surgery. Mice were anesthetized with isoflurane (3%) and maintained under anesthesia (2 %) throughout the surgery. The AAV vector was injected bilaterally into the DG hilus (stereotactic coordinates AP: 2.1mm, ML: ±1.5mm, DV: -2.1mm relative to bregma) at a rate of 120 nl/min (1,000nl per side), and the needle was left in place for an additional 8 mins to permit diffusion. The control groups were injected with an AAV5-EF1α-mCherry virus (VN2098 from Vector Biolabs). All surgical procedures were performed under aseptic conditions. Mice were kept warm on a heating pad as they recovered. Mouse chow moistened with water was placed in the cage once they recovered to encourage eating and provide hydration following surgery. To allow time for recovery and viral expression, animals were housed for three weeks after injection and before any behavioral experiments were initiated. Injection locations were verified histologically at the end of the study.

Behavioral Tests

All behavioral tests were performed in the morning, with at least 1 h of habituation in the behavioral room prior to experimentation on each day.

Novel Object Recognition Test

Mice were habituated in the experimental chamber for two days before the testing phase and each habituation period lasted for 15 min for each animal per session. During the training phase on day three, the mouse was allowed to explore two copies of the same object for 10 min. The mouse was then returned to its home cage for a retention period of 1 h. The mouse was reintroduced to the training context and presented with one familiar sample object and one novel object for 10 min. Movement and interaction with the objects were recorded with the EthoVision XT video-tracking system, which was mounted above the chamber, and exploratory behavior was measured.
Interaction time was recorded using the multiple body point module. Memory was assessed using a the recognition index (i.e., the ratio of time spent exploring the novel object to the time spent exploring both objects). After each mouse had performed the test, the chamber and objects were swabbed thoroughly with alcohol to avoid any interference with tests involving subsequent mice.

Morris Water Maze and Reversal Learning

The spatial learning and memory function of rats were assessed using the Morris water maze. The test lasted for 10 days: Learning phase Session 1-4 (day1-4); Learning phase probe trials – Session 5 (Day 5); Reversal learning phase Session 6-9 (day 6-9) & Reversal learning probe trials- Session 10 (day10). Mice are tested in a pool (120 cm in diameter) filled with water (23-25°C) made opaque with the addition of a white nontoxic dye (Premium Grade Tempera, Blick, Galesburg, IL) containing a platform (10 cm in diameter) that is submerged by 1 cm under water surface. Geometric shapes are affixed to the walls to serve as extra-maze cues. Mice are given 3 trials every day released from a different quadrant each time in random order, with the platform location constant. A trial ends either 2 sec after the mouse climbs on the platform, or 60 sec after the start of the trial, with the experimenter guiding the mouse to the platform. If a mouse could not find the platform within 60 sec it was gently guided to the platform and the escape latency was recorded

as 60 sec. After the mouse reached the platform, it was allowed to stay there for 10 seconds before the next trial was performed. From day 6 to day 9, the reversal learning phase was established by moving the platform from the original location to the nearest quadrant to increase the effects of interference. During probe trial (day 5) and reversal learning probe trial (day 10), the platform was removed, and the mice were left in the pool for 60 seconds. The track of mice was recorded using a camera positioned above the center of the pool and connected the EthoVision XT software video-tracking system.

Y-maze test

Spontaneous alternation in the Y maze was tested as a measure of spatial working memory. The apparatus was a Y-shaped maze with three gray, opaque plastic arms at a 120° angle from each other. The arm dimensions were 15 cm (height) x 30 cm (length) x 8 cm (width). Each animal was placed in the center of a symmetrical Y-maze and was allowed to explore freely during an 8-min session. The sequence and total number of arms entered were recorded with a video camera that was mounted above the apparatus. Arm entry was defined as entry of the whole body into an arm. Alternation was defined as successive entries (ABC, ACB, BCA, BAC, CBA, CAB) into the three arms in overlapping triple sets. The % of alternation was calculated as the number of triads containing entries into all three arms divided by the maximum possible number of alternations (the total number of arms entered − 1) × 100. To diminish odor cues, the Y maze was cleaned with 70% ethanol between trials and allowed to dry.

Biochemical and molecular parameters

Mice were deeply anesthetized with ketamine (139 mg/kg i.p.) / xylazine (21 mg/kg i.p.) and then transcardially perfused with ice-cold phosphate-buffered saline (PBS) followed by 4% ice-cold paraformaldehyde. After 24 hours of post-fixation at 4°C, brains were moved into 30% sucrose for 72 hours and sectioned into 30 µm coronal sections using a cryostat. Free-floating sections were collected mounted on slides for staining.

Immunofluorescence staining

Immunofluorescence staining was used to validate hilar SOM+ interneuron manipulation. Free-floating coronal sections (30 μm) were nuclei stained with DAPI for 10 min in the dark, and the sections were transferred to glass slides and mounted with Anti-Fade Fluorescence Mounting Medium (Vector Laboratories). Images were acquired with a Leica microscope (Leica, Germany).

Immunohistochemistry

We performed analysis of sections for target cell labeling and quantification. For analysis of c-Fos staining, all mice (n=6 per group) were exposed to a novel environment for 60 min and then placed into a clean cage individually for 1 hour before perfusion. Free-floating coronal sections (30 μm) were incubated in blocking solution (2% normal goat serum and 0.4% Triton X-100 in PBS) for 2 h at room temperature and then with primary antibodies at 4°C overnight. The primary antibodies used were as follows: rabbit anti-c-Fos (Cell Signaling Tech, cat # 2250, 1:500), anti-somatostatin (SOM, Invitrogen, cat # PA5-82678, 1:1000) and anti-glutamic acid decarboxylase 67 (GAD-67, Invitrogen, cat # PA5-21397, 1:500). After washing with blocking solution, we added 3% H2O2 for 10 min at room temperature. After washing with blocking solution, the sections were incubated in a biotinylated goat anti-rabbit secondary antibody in blocking solution (Invitrogen, cat # 31820, 1:500) and transferred in a detection reagent (Vecstatin Elite ABC kit, Vector Laboratories, cat # PK-7200). Immunoreactivity was visualized using the VECTASTAIN ABC kit (Vector) for 30 min and Vector NovaRED (Vector) as the chromogen for 3 min at room temperature. Finally, brain sections were washed with distilled water, transferred to glass slides, dehydrated in 95% and 100% ethanol for 1 min each and mounted with Eukitt mounting medium (Sigma-Aldrich).

Statistical Analysis

Statistical analyses were performed (GraphPad Prism) using 1-way ANOVA followed by Tukey test for multiple comparisons or two-tailed t-test. Results are represented as mean ± S.E.M. and P values, are specified in each figure legend. P values less than 0.05 were considered significant.

Results

In order to test whether a partial ablation of SOM+ interneurons induce an aging-related phenotype, we stereotactically injected AAV5-EF1α-mCherry-flex-dtA into the DG hilus of Sst-IRES-Cre mice and starting 21 days later with behavioral experiments, followed by immunohistochemical analysis (Fig. 1). To validate the specificity of the hilar SOM+ interneuron manipulation, we performed immunofluorescence staining of coronal sections from experimental mice with DAPI counterstain and mCherry stain (Fig. 2). With the viral vector used, the mCherry expression should occur in every transduced cell in which the EF1α promoter is active and independently of Cre-*lox*P-mediated recombination, whereas the expression of the diphteria toxin subunit A is dependent on Cre-*lox*P-mediated recombination.

Partial ablation of hilar SOM+ interneurons leads to increased c-Fos+ staining in DG and CA3.

Hilar-perforant-path-associated interneurons (HIPP cells) in the DG hilus are SOM+ cells that distribute axon fibers in the molecular layer, providing dendritic inhibition of DG granule cells(Yuan et al., 2017). We hypothesized that partial ablation of SOM+ neurons in the DG hilus would thus increase the activity in DG granule cells. Indeed, c-Fos staining in DG hilus ($p<0.001$), DG granule cell layer (GCL) ($p<0.001$), and total DG ($p<0.001$) was increased compared to controls, consistent with hyperactivity. c-Fos staining in the CA3 subregion of the hippocampus was also increased ($p<0.001$), whereas c-Fos staining in the CA1 subregion of the hippocampus was unchanged ($p>0.05$) (Fig.3).

Partial ablation of hilar SOM+ interneurons leads to decreased SOM+ and GAD-67+ neurons

We then assessed the consequences of the ablation of SOM+ interneurons on the expression of two interneuronal markers. In mice with partial ablation of SOM+ interneurons, the number of SOM+ cells was reduced in DG ($p<0.05$) and CA3 ($p<0.05$) compared to controls, but not in CA1

($p>0.05$) (Fig 4). In addition, the number of GAD-67+ was significantly decreased only in DG hilus of mice with partial ablation of SOM+ interneurons ($p<0.01$), whereas in CA3 ($p>0.05$) and CA1 ($p>0.05$), no significant change was observed for GAD-67+ staining (Fig. 5). These results indicate that our experimental manipulation led to an approximately 50% reduction of SOM+ interneurons in the DG and also in the number of GAD-67+ interneurons.

Partial ablation of hilar SOM+ interneurons induced recognition memory impairment in the NOR test

We investigated the effect of partial ablation of SOM+ interneurons on recognition memory in the NOR test. This test is based on the natural tendency of mice to explore a novel object rather than a familiar object. Mice with partial ablation of SOM+ interneurons preferred novel objects over familiar objects to a lesser degree than control mice, which resulted in a significant decrease in the recognition index ($p<0.05$) compared to controls, indicating that recognition memory is impaired in these mice (Fig. 6A). We observed no significant differences between males and females in the NOR Test (data not shown).

Partial ablation of hilar SOM+ interneurons induced working memory impairment in the Y maze test

The Y-maze task was used to assess short-term working memory. Mice with intact working memory would run in the three arms with spontaneous alternations. We found that mice with partially ablated SOM+ interneurons the D/A injection displayed a reduction in the percentage of correct alternations compared to control mice ($p<0.05$), indicating that short-term working memory is impaired (Fig.6B). We observed no significant differences between males and females in the Y maze (data not shown).

Partial ablation of hilar SOM+ interneurons induced spatial learning and memory impairment

To investigate the potential spatial memory deficits in the experimental mice, we performed the Morris Water Maze (MWM). During the learning phase, the control mice displayed a significantly shorter latency to reach the platform on day 4 compared to day 1 ($p<0.05$), while the partial

ablation mice did not show any improvement ($p>0.05$), which resulted in a difference between the two groups on day 4 ($p<0.01$) (Fig. 7A). The path length in the control mice was reduced on days 3 and 4 ($p<0.05$), whereas in partial ablation mice there was no change compared to day 1 ($p>0.05$), resulting in a difference between the groups on day 4 ($p<0.01$) (Fig. 7B). In the learning phase probe trials on day 5, the partial ablation group spent a reduced cumulative duration in the platform area compared to controls ($p<0.05$) (Fig. 7C) and also displayed a reduced frequency to enter the platform position ($p<0.05$) (Fig. 7D).

On day 6, the position of the hidden platform was changed to assess reversal learning. In control mice, the latency to find the platform was reduced on days 8 ($p<0.05$) and 9 ($p<0.05$) compared to day 6, whereas there was no change in the latency to find the platform in the partial ablation mice ($p>0.05$). The latencies of both groups were significantly different on days 7 ($p<0.05$), 8 ($p<0.01$) and 9 ($p<0.01$) (Fig. 7A). In the same trials, the path length of control mice ($p>0.05$) and partial ablation mice ($p>0.05$) was unchanged compared to day 6. However, both groups differed on day 9 with the partial ablation mice displaying an increased path length ($p<0.05$) compared to the control group (Fig. 7B). In the reversal learning phase probe trials on day 10, both groups of mice did not differ in the cumulative duration spent in the platform area ($p>0.05$) (Fig. 7E) and in the frequency to enter the platform position ($p>0.05$) (Fig. 7F).

Overall, our results indicate that during the learning phase – and to a lesser degree during the reversal learning phase – the partial ablation of SOM+ interneurons in the DG results in cognitive impairments.

Discussion

In this study, we investigated the consequences of partial ablation of SOM+ interneurons in the DG hilus for hippocampal learning and memory. We found that our experimental approach led to an approximatly 50% reduction of the number of SOM+ interneurons in the DG. This resulted in increased c-Fos staining in DG and CA3, and in cognitive impairments in the novel object recognition memory, the Y maze and the MWM with learning and reversal learning phases. Our work shows that partial ablation of DG hilar SOM+ interneurons is sufficient to elicit a phenotype that is remarkably similar to that of aged mice. The mice with partial ablation of DG hilar SOM+ interneurons potentially represent a cellularly defined model of hippocampal aging.

A remarkable finding was thae increase f-Fos staining in DG and CA3, as hyperactivity in DG and CA3 also appears to be a hallmark of aging Click or tap here to enter text.. The c-Fos gene is a cellular proto-oncogene that can be triggered by neuronal activation, cAMP pathway stimulation, and Ca^{2+} elevation in the brain(Hudson, 2018). Here, we used c-Fos as a marker for neuronal activity in the hippocampus and found increased c-Fos expression in the Total DG, DG hilus, DG GCL layer and CA3 in the partial ablation mice. Thus, increased c-Fos-activity in DG and CA3 is associated with the cognitive impairment the partial ablation mice. The c-Fos staining results in the SOM+ ablation model are similar to the ones that we have previously observed in mice lacking α5-$GABA_A$ receptors in dentate granule cells(Engin et al., 2015), suggesting that a reduction of inhibition of the DG granule cells by hilar SOM+ perforant path-associatd (HIPP) neurons(Yuan et al., 2017) may play an important role in this model.

Ablation of SOM+ interneurons in the DG hilus led to impaired performance in the Y maze alternation test, assessing working memory, in the novel object recognition (NOR) test, assessing recognition memory, in initial learning in the Morris water maze (MWM), assessing spatial learning, and in the reversal phase of the MWM, assessing reversal learning. As in our partial ablation mice the experimental manipulation is restricted to the hippocampus, it is reasonable to assume that the behavhioral deficits that we see are of hippocampal origin. Based on studies on the role of α5-$GABA_A$ receptors in CA1 and DG (Engin et al., 2015, 2020), initial learning in the MWM is largely CA1-dependent and reversal learning largely DG-dependent. Our results suggest

that ablation of SOM+ interneurons in the DG – like aging – results in cognitive deficits that are due to DG and CA1 dysfunction. The cognitive deficits that we saw in the partial ablation mice were relatively similar to the cognitive decline that we saw in 18 months old mice compared to 3-4 months old mice (not shown).

The decreased number of SOM-positive and GAD-67-positive interneurons in the hippocampus of partial ablation mice reported here is consistent with findings in other rodent models of aging. Andrews-Zwilling et al. (2010) reported that apoE4 knock-in (KI) mice had an age-dependent decrease in hilar GABAergic interneurons that correlated with the extent of learning and memory deficits, as determined in the Morris water maze in aged mice, which is similar to our findings in mice with partial ablation. Treating apoE4-KI mice with the $GABA_A$ receptor potentiator pentobarbital rescued the learning and memory deficits. In neurotoxic apoE4 fragment transgenic mice, hilar GABAergic interneuron loss was even more pronounced and also correlated with the extent of learning and memory deficits (Andrews-Zwilling et al., 2010). Spiegel et al. (2013) reported that the hilar SOM-positive interneurons exhibit an age-dependent change in numbers in outbred Long-Evans rats, with significantly reduced numbers in the subpopulation of aged rats with behavioral impairment while unimpaired aged rats had SOM+ interneuron numbers that did not differ from young adults(Spiegel et al., 2013). Interestingly, postmortem studies of patients with AD have shown reduced SOM expression in the hippocampus and cortical areas (Beal et al., 1985; Davies et al., 1980; Dournaud et al., 1994), and the decreased expression was correlated with the severity of cognitive deficits (Dournaud et al., 1995) and neuropathology(Dawbarn et al., 1986). The current findings of memory impairments in our partial ablation mice are therefore consistent with other evidence in animal models and studies in humans indicating that a loss of hilar integrity may be underlying such an age-related memory impairment. Our findings are also consistent with a potential contribution of GABAergic interneuron impairment to the pathogenesis of AD.

It is important to note that all SOM-expressing hilar interneurons colocalize GAD-67(Freund & Buzsáki, 1998; Houser, 2007). The current data show that the decline in SOM-expressing hilar interneurons accounted almost entirely for the magnitude of hilar GAD67-positive neuron loss observed in partial ablation mice. This strongly suggests that hilar vulnerability under cognitive impairment during aging predominantly involves the GABAergic hilar interneurons that are also

positive for SOM. Decreased GAD-67 and SOM protein expression may impact hilar interneuron function in partial ablation mice. While other approaches would be needed to fully document the physiological consequences of hilar interneurons lacking SOM and GAD-67 expression, evidence suggests that reduced levels of GAD-67 protein expression would result in diminished functional inhibition. In support of this possibility, Lau and Murthy (2012) reported that decreased levels of cytosolic GAD-67 expression are associated with reduced GABA synthesis, diminished GABA release at the synapse, and decreased miniature inhibitory postsynaptic current amplitudes (mIPSCs) in cultured hippocampal neurons(Lau & Murthy, 2012). The concept that reduced inhibitory function in the hippocampal memory network contributes to cognitive dysfunction, as suggested by our findings, is consistent with the presence of hyperactivity of the DG and CA3 subregions in both humans and animal models with age-related memory impairment (Bakker et al., 2012; Wilson, 2005; Yassa et al., 2011). Moreover, targeting that hyperactivity has been found to improve hippocampal-dependent memory function(Andrews-Zwilling et al., 2010; Bakker et al., 2012; Koh et al., 2013).

In conclusion, our results show that the partial ablation of hilar SOM-positive interneurons is sufficient to induce changes similar to those observed in aged rodents, such as reduction of the SOM and GAD-67 markers in the DG hilus, hyperactivity in DG and CA3 of the hippocampus and cognitive impairments. This suggests that the changes elicited by partial ablation of hilar SOM-positive interneurons may contribute to the development of cognitive dysfunction in aging animals. Furthermore, mice with a partial ablation of hilar SOM+ interneurons may represent a model for studying mechanisms underlying cognitive decline in aging.

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Declaration of Interests

The authors declare no competing interests.

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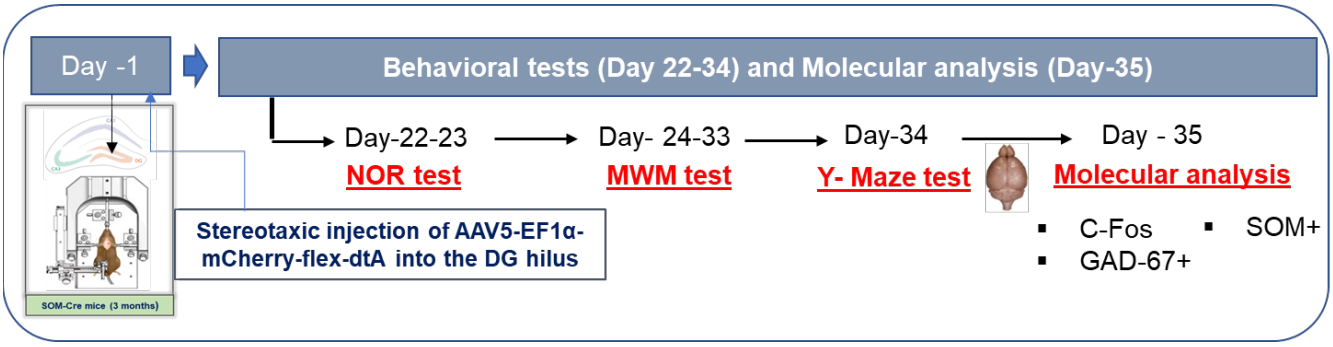

Fig. 1: Schematic overview of the experimental protocol. NOR - Novel Object Recognition test ; MWM - Morris water maze; SOM+ - Somatostatin-positive interneurons and GAD-67+ - Glutamic acid decarboxylase 67-positive interneurons.

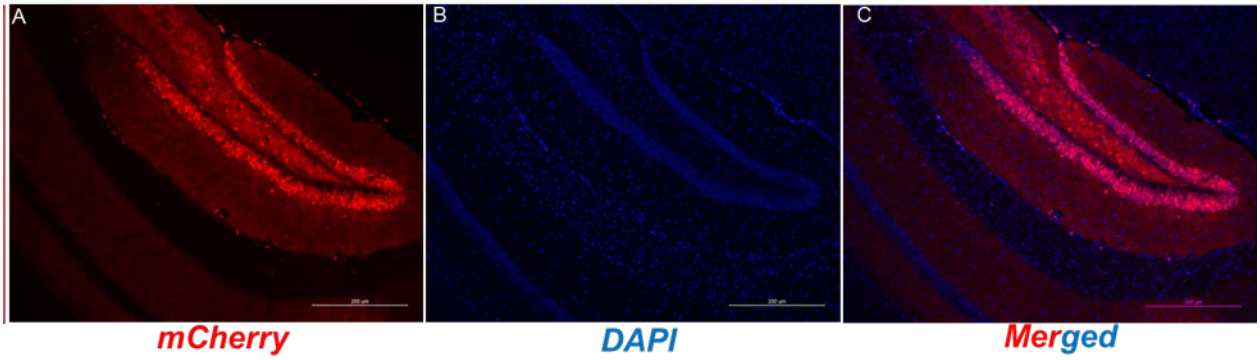

Fig. 2: Validation of viral injection into the DG hilus. The viral vector AAV5-EF1α-mCherry-flex-dtA was injected into the DG hilus of SOM-Cre mice. Three weeks later, the mice were perfused. Slices were stained with DAPI. The red mCherry fluorescence is shown in A, DAPI staining is shown in B, and the superimposition of A and B shown in C.

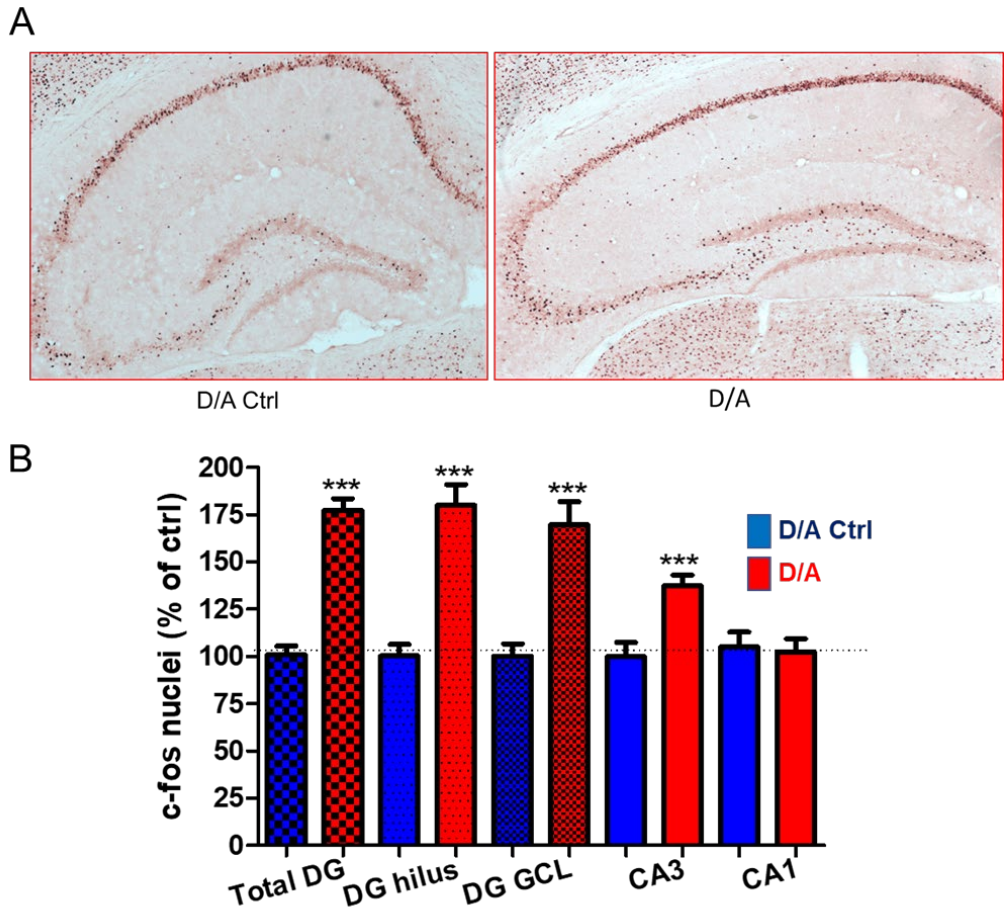

Fig. 3: Partial ablation of Hilar SOM+ interneurons increased c-fos+ neurons in the DG & CA3 region of the hippocampus. *A)* Representative microscopical images of neurons positive for c-fos. B) Histograms show the estimated density of c-fos+ cells in D/A mice expressed as a percentage of D/A control mice. The data were analyzed using NIH Image J software. ***$p<0.001$ vs D/A Ctrl, (n=4). **D/A Ctrl** – (Control) injected with an AAV5-EF1α-mCherry virus; **D/A**- injected with AAV5-EF1α-mCherry-flex-dtA; **DG** - Dentate gyrus, and **GCL**- DG granule cell layer. D/A - Diphtheria toxin A.

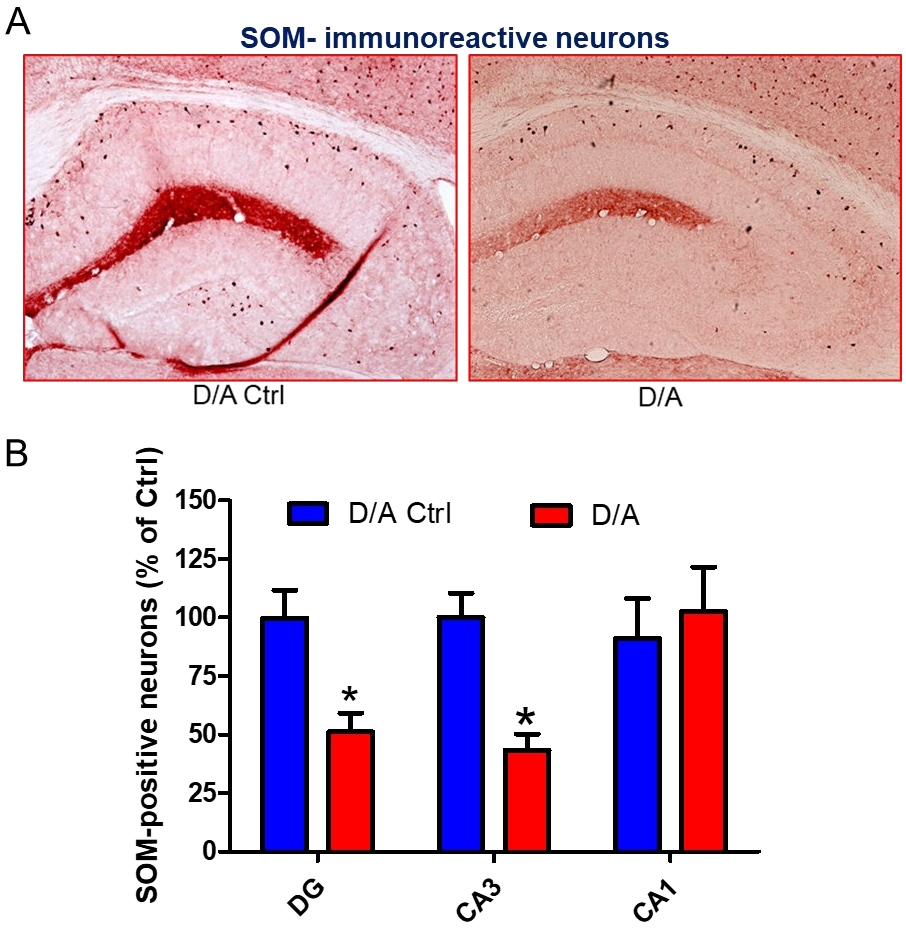

Fig. 4: Immunohistochemical staining of SOM+ after partial ablation of Hilar SOM+ interneurons. A) Representative microscopical images of neurons positive for SOM. B) Histograms show the estimated density of SOM+ cells in mice with partial ablation of SOM+ interneurons and controls. The data were analyzed using NIH Image J software. *$p<0.05$ vs D/A Ctrl, (n=4). D/A - Diphtheria toxin A.

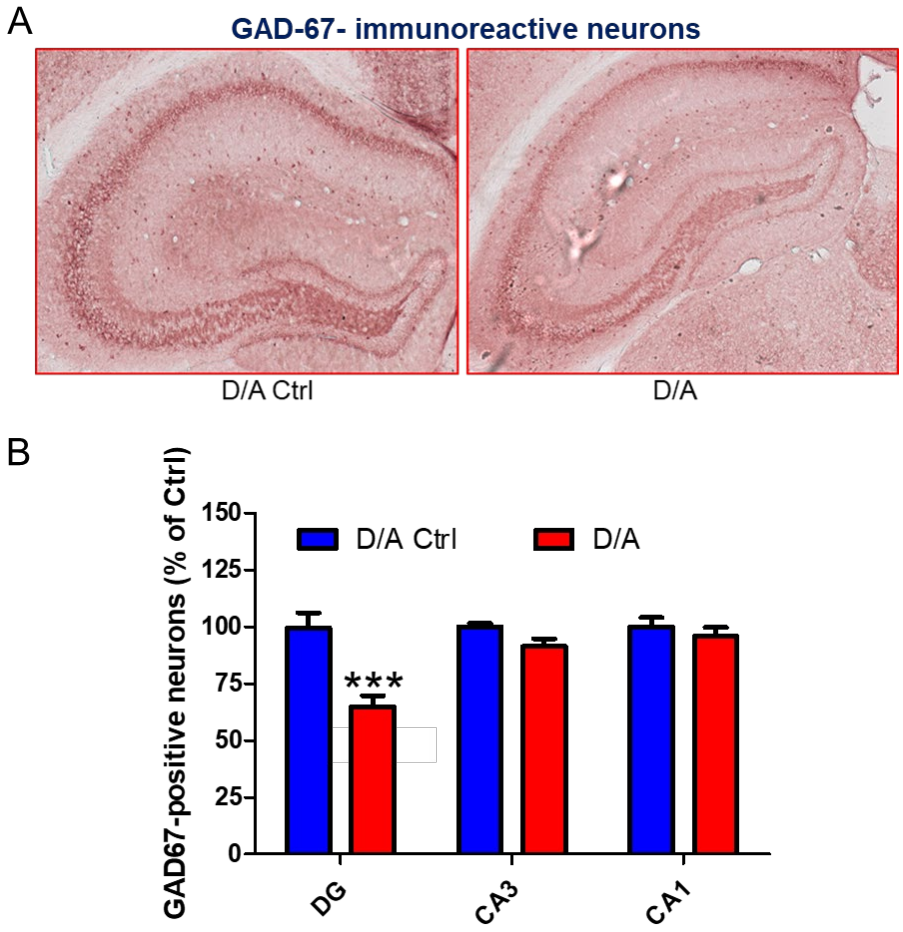

Fig. 5 Immunohistochemical staining of SOM+ after partial ablation of Hilar SOM+ interneurons. A) Representative microscopical images of neurons positive for *GAD-67*. B) Histograms show the estimated density of *GAD-67*+ cells in D/A partial ablation mice expressed as a percentage of D/A control mice. The data were analyzed using NIH Image J software. ***$p<0.001$ vs D/A Ctrl, (n=4). D/A - Diphtheria toxin A.

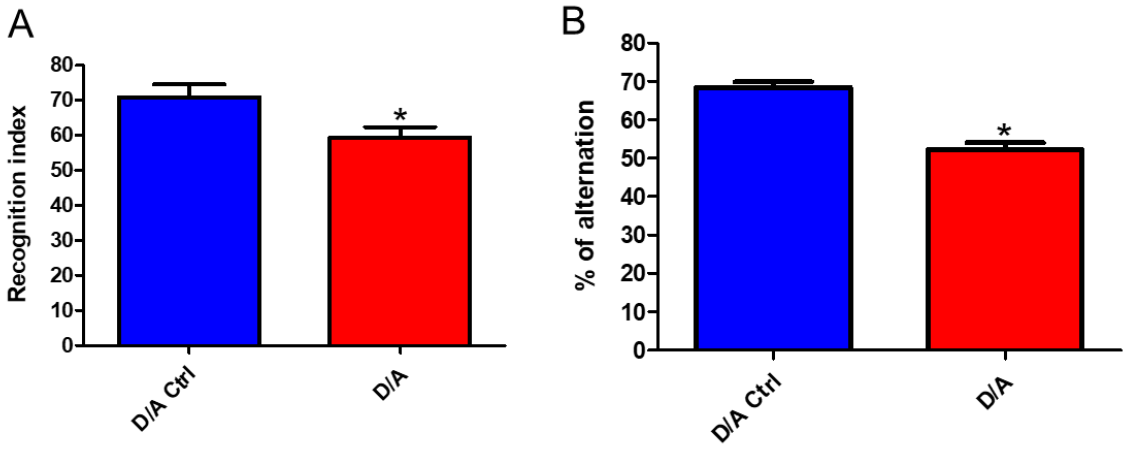

Fig. 6: Novel object recognition test and Y maze test in mice with partial ablation of hilar SOM+ interneurons. A) Recognition index in the Novel Object Recognition test (NOR) and **B)** Spontaneous arm alternation percentage in the Y-maze test. Results are expressed as mean ± SEM. *$p<0.05$ vs D/A Ctrl, (n=14-16). D/A - Diphtheria toxin A.

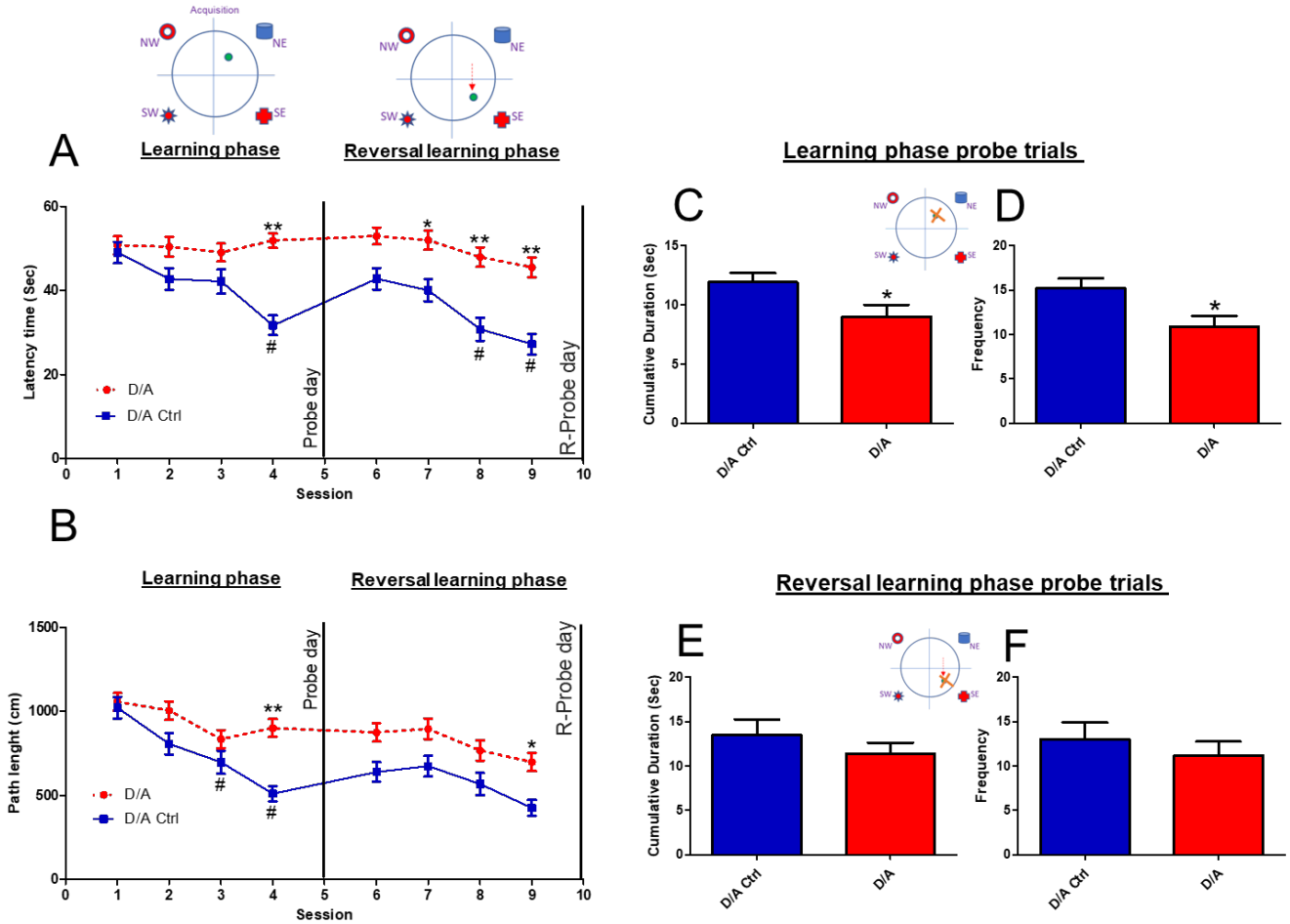

Fig. 7: Morris water maze test with learning and reversal learning phases in mice with partial ablation of hilar SOM+ interneurons. Learning phase Session 1-4 (day1-4); Learning phase probe trials – Session 5 (Day 5); Reversal learning phase Session 6-9 (day 6-9) & Reversal learning probe trials – Session 10 (day 10). A) Latency time (sec) and B) Path length (cm) to the hidden platform in the learning phase (*p<0.05 vs D/A Ctrl & #p<0.05 vs Session 1 - D/A Ctrl) and Reversal learning phase *p<0.05, **p<0.01 vs D/A Ctrl & #p<0.05 vs Session 6 - D/A Ctrl). C) Cumulative duration and D) Frequency to enter target platform area in learning phase probe trials *p<0.05 vs. D/A Ctrl, (n=14-16). E) Cumulative duration and F) frequency to enter the target platform area in the reversal learning phase of probe trials. R-Probe day - Reversal learning probe trials. D/A - Diphtheria toxin A.