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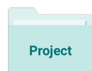

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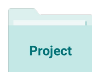

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Hippocampal extracellular matrix alterations contribute to cognitive impairment associated with a chronic depressive-like state in rats

Danai Riga[1], Ioannis Kramvis[2†], Maija K. Koskinen[1†], Pieter Van Bokhoven[1†], Johanneke E. Van der Harst[3,4], Tim S. Heistek[2], A. Jaap Timmerman[2], Pim Van Nierop[1], Roel C. Van der Schors[1], Anton W. Pieneman[1], Anouk de Weger[1], Yvar Van Mourik[5], Anton N.M. Schoffelmeer[5], Huib D. Mansvelder[2], Rhiannon M. Meredith[2], Witte J.G. Hoogendijk[6], August B. Smit[1‡], Sabine Spijker[1‡*]

Affiliations:

[1]Dept. of Molecular & Cellular Neurobiology, Center for Neurogenomics & Cognitive Research, Amsterdam Neuroscience, VU University, Amsterdam, The Netherlands.

[2]Integrative Neurophysiology, Center for Neurogenomics & Cognitive Research, Amsterdam Neuroscience, VU University, Amsterdam, The Netherlands.

[3]Dept. Biology, Institute of Environmental Biology, Animal Ecology group Biology, Utrecht University, The Netherlands.

[4]Dept. Cognitive Neuroscience, Radboud University Medical Center, Nijmegen, The Netherlands.

[5]Dept. of Anatomy and Neurosciences, Amsterdam Neuroscience, VU Medical Center, Amsterdam.

[6]Dept. of Psychiatry, Erasmus Medical Center, Rotterdam, The Netherlands.

*s.spijker@vu.nl

[†]Shared second authors; [‡]Shared senior authors

Abstract

Patients with depression often suffer from cognitive impairments that contribute to disease burden. We used social defeat-induced persistent stress (SDPS) to induce a depressive-like state in rats and then studied long-lasting memory deficits in the absence of acute stressors in these animals. The SDPS rat model showed reduced short-term object location memory and maintenance of long-term potentiation (LTP) in CA1 pyramidal neurons of the dorsal hippocampus. SDPS animals displayed increased expression of synaptic chondroitin sulfate proteoglycans in the dorsal hippocampus. These effects were abrogated by a 3-week treatment with the antidepressant imipramine starting 8 weeks after the last defeat encounter. Next, we observed an increase in the number of perineuronal nets (PNNs) surrounding parvalbumin-expressing interneurons and a decrease in the frequency of inhibitory postsynaptic currents (IPSCs) in the hippocampal CA1 region in rats exposed to SDPS. In vivo breakdown of the hippocampus CA1 extracellular matrix by the enzyme chondroitinase ABC administered intracranially restored the number of PNNs, LTP maintenance, hippocampal inhibitory tone, and memory performance on the object place recognition test. Our data reveal a causal link between increased hippocampal extracellular matrix and the cognitive deficits associated with a chronic depressive-like state in rats exposed to SDPS.

Introduction

Major depressive disorder (MDD) is a complex neuropsychiatric disorder that is characterized by persistent negative mood, a multifaceted anhedonic state, and impaired cognitive function (*1*). MDD is considered one of the leading causes of disability worldwide, accounting for more lost productivity than any other psychiatric disorder (*1*). A substantial part of this burden is attributed to the cognitive impairment that accompanies depression, including deficits in working and episodic memory (*2*), which could persist beyond recovery from mood disturbances (*3*). Despite compelling evidence linking these deficits to reduced hippocampal volume (*4*) and impaired hippocampal function (*5*), the molecular basis underlying the effects of MDD on cognition remains unclear.

Persistent stress responses, commonly triggered by stressful life events, are a potent causal factor in eliciting MDD (*6*) and have major repercussions for hippocampal function (*7*). In line with this, preclinical models of depression using acute stress consistently show hippocampal pathology, including reduced hippocampal long-term potentiation (LTP) and impaired hippocampus-mediated spatial learning (*8*, *9*). In contrast, the chronic phase of depression in the months after initial stress exposure has only been scarcely explored, posing questions about the underlying neurobiological mechanisms.

In an attempt to address this issue, we adopted the social defeat– induced persistent stress (SDPS) rat model in which a sustained depression-like state was elicited by exposure to five daily defeat episodes and individual housing for a period of 2 to 3 months in the absence of acute stressors (*10*). Previously, the SDPS model has allowed us to investigate sustained affective and cognitive deficits on a variety of behavioral tests (*11*, *12*). Here, we investigated the underlying

mechanisms of cognitive dysfunction triggered by the chronic depressive-like state of rats exposed to SDPS (*11*, *12*).

Results

SDPS induces imipramine-reversible deficits in hippocampus-mediated memory

We assessed the effects of a chronic depressive-like state on memory performance in rats exposed to the SDPS paradigm. We then examined the potentially restorative action of the tricyclic antidepressant drug imipramine (Fig. 1A). First, we confirmed that physiological F1 (corticosterone) and behavioral adaptations (body weight and food intake) in response to acute social stress had completely subsided 8 weeks after the last defeat exposure (fig. S1) (*13*). We then evaluated cognitive capacity using the object place recognition (OPR) test and novel object recognition (NOR) test, which assess short-term object location (spatial) and recollection memory, respectively (*14*, *15*).

SDPS impaired the retention of spatial information [$P = 0.044$ versus vehicle-treated (H2O) control] expressed as reduced exploration of the displaced object during the test phase of the OPR task (Fig. 1B). Vehicle-treated control animals displayed a clear preference for the displaced object [control-H2O, $P = 0.041$ versus a fictive control showing no discrimination (exploration index 0.50), while retaining the variation of the tested sample] (*16*). In contrast, SDPS rats displayed no such preference (SDPS-H2O, $P = 0.478$ versus fictive control), indicating a reduced ability to retain short-term memories. Oral imipramine administration during the last 3 weeks of the SDPS paradigm (Fig. 1A), previously shown to ameliorate SDPS-induced hippocampal pathology (*13*, *17*), normalized performance on the OPR test [two-way analysis of variance (ANOVA), group × treatment interaction effect, $P = 0.014$; post hoc SDPS-H2O versus control-H2O, $P = 0.044$; SDPS-imipramine versus control-imipramine, $P = 0.122$; SDPS-H2O versus SDPS-imipramine, P

= 0.010] (Fig. 1B). SDPS had no effect on the performance on the NOR test ($P = 0.819$ versus control-H2O); both groups showed a preference for the novel object (control-H2O, $P = 0.005$; SDPS-H2O, $P = 0.005$ versus fictive control) (Fig. 1C). Similarly, treatment with the antidepressant imipramine did not affect the performance on the NOR test in either group (two-way ANOVA, group × treatment, $P = 0.379$) (Fig. 1C).

Given that the optimal performance on the OPR test requires an intact dorsal hippocampus (*18*), we assessed the effects of the SDPS paradigm on synaptic plasticity in the dorsal hippocampus. SDPS reduced maintenance of LTP in the hippocampal CA1 subfield (0.8-fold; $P = 0.001$ versus control-H2O; fig. S2), and imipramine treatment reversed this effect ($P = 0.937$ versus control-imipramine), as previously reported (*17*). Thus, the SDPS paradigm promoted an enduring depressive-like state in rats that was characterized by a reduction in hippocampal plasticity and deficits in short-term object location memory; these deficits were ameliorated by antidepressant treatment. Notably, individual housing alone devoid of the social defeat stress component did not affect the performance on the OPR test or LTP maintenance (fig. S2), indicating that SDPS specifically affected hippocampal function.

SDPS induces an imipramine-reversible increase in synaptic chondroitin sulfate proteoglycans

We next investigated SDPS-induced changes in the dorsal hippocampal synaptic proteome that might underlie the observed perturbations in plasticity and memory. These effects were not mediated by changes in the expression of AMPA or NMDA receptors (*19*) in the synaptic membrane fraction or by global changes in the number of glutamatergic or GABAergic synapses, as reflected by no change in PSD-95 or gephyrin expression (fig. S3). Therefore, we examined whether SDPS induced unique imipramine-reversible changes in protein expression in the rat

1 hippocampus. For this, we used an unbiased differential proteomics analysis of the dorsal
2 hippocampal synaptic membrane fraction ($n = 5$).
3 From a total of 519 proteins identified by mass spectrometry (≥2 distinct peptides;
4 confidence interval, ≥95%), 37 proteins were significantly regulated by SDPS ($P < 0.05$; adjusted
5 for multiple testing) (*20*). The expression of a subset of 18 proteins was restored by imipramine
6 treatment. Overrepresentation analysis using gene ontology (GO) annotation (*21*) revealed a large
7 contribution of extracellular matrix proteins both in the total set (adjusted $P = 0.039$; Fig. 2A) and
8 among proteins whose expression was rescued by imipramine (adjusted $P = 0.025$; Fig. 2B) (table
9 S1). In particular, SDPS increased the expression of chondroitin sulfate proteoglycans (CSPGs;
10 table S1), glycosaminoglycan-carrying lecticans that are considered to be major constituents of
11 adult brain extracellular matrix (*22*). CSPGs reside in the perisynaptic space at contact sites with
12 astrocytes, actively contributing to the tetrapartite synaptic complex (*23*, *24*). CSPGs assemble
13 into pericellular netlike formations that envelop interneurons, the so-called perineuronal nets
14 (PNNs) (*25*).
15 In an independent group of animals ($n = 4$ to 5), we investigated the expression of seven
16 core components of adult brain extracellular matrix, namely, the CSPGs aggrecan, brevican,
17 neurocan, phosphacan, and versican. We also looked at the expression of the proteins tenascin-R
18 and hyaluronan and proteoglycan link protein 1 (HPLN1), which contribute to the assembly of
19 PNNs, in the synapse-enriched fraction of the dorsal hippocampus. Quantitative immunoblotting
20 confirmed the SDPS-induced increase in the expression of brevican (twofold; $P = 0.016$), neurocan
21 (twofold; $P = 0.001$), phosphacan (1.9-fold; $P = 0.010$), and HPLN1 (1.8-fold; $P = 0.009$),
22 compared to vehicle-treated control rats (Fig. 2, C to F, and fig. S4). SDPS had a modest but
23 nonsignificant effect on the expression of tenascin-R (1.8-fold; $P = 0.068$), aggrecan (1.5-fold; P

1 = 0.139), and versican (1.3-fold; P = 0.181) (Fig. 2, G and H, and fig. S4). Imipramine treatment
2 reversed SDPS-induced changes in CSPG expression and no significant differences between the
3 two imipramine-treated groups (control-imipramine versus SDPS-imipramine) were detected
4 (brevican, P = 0.230; neurocan, P = 0.443; phosphacan, P = 0.284; HPLN1, P = 0.251) (Fig. 2, C
5 to F). Aberrant CSPG expression was specific to the hippocampal synaptic membrane fraction
6 because no increase in CSPG expression was detected in the tissue lysates collected before
7 isolation of synaptic membranes (fig. S5). Together, these data establish that SDPS specifically
8 alters the composition of perisynaptic extracellular matrix in the dorsal hippocampus and that
9 imipramine reverses this effect.
10
11 *SDPS increases the number of PNNs and decreases inhibitory transmission in the hippocampal*
12 *CA1 region*
13 We next examined whether SDPS affected the organization of CSPG-rich PNNs in the dorsal
14 hippocampus (*26*). Immunohistochemical analysis (Fig. 3A and fig. S6) showed that the number
15 of PNN-coated neurons was increased after SDPS (1.6-fold; P = 0.032 versus control), specifically
16 in the CA1 subfield of the dorsal hippocampus (Fig. 3B). Characterization of these PNN-coated
17 neurons in an independent set of animals revealed that this increase was unique to parvalbumin-
18 expressing interneurons located in the CA1 stratum pyramidale of the hippocampus (1.4-fold; P =
19 0.044 versus control; Fig. 3C and fig. S7), where the vast majority (>90%) of PNN-associated cells
20 are parvalbumin-positive (fig. S8). This was in the absence of changes in the overall intensity of
21 PNN immunostaining (Fig. 3D and fig. S7). No group difference in the number of PNN-coated
22 parvalbumin-negative neurons (P = 0.146) was detected (Fig. 3C). SDPS had no effect on the
23 number of PNNs located in the stratum oriens, where a much lower percentage (~50%) of PNN-

coated parvalbumin-positive interneurons was identified (fig. S8). Finally, in accordance with our behavioral data showing an absence of SDPS effects on the NOR test (Fig. 1C), we detected no SDPS-induced changes in the number of PNN-coated neurons in the perirhinal cortex (fig. S9) (*27*).

PNNs are known to alter the structural and physiological properties of parvalbumin-positive neurons (*28*, *29*). Therefore, we examined the effects of SDPS on CA1 stratum pyramidale interneuron morphology and their excitatory synaptic input. SDPS did not affect the total number of PNN-coated parvalbumin-positive inter neurons ($P = 1.00$; Fig. 4A) but increased the intensity of parvalbumin immunoreactivity in these neurons (8%; $P = 0.008$; Fig. 4B). A significant reduction (−10% versus control; $P = 0.043$) in the fraction of cells with intermediate-low parvalbumin expression was observed after SDPS, which coincided with an increase in the fraction of interneurons with high expression of parvalbumin (17% versus control; $P = 0.002$; Fig. 4, C and D). Notably, this intensity shift was not observed in parvalbumin-positive neurons that were not PNN-coated (Fig. 4D). Increased parvalbumin immunostaining has been associated with reduced structural synaptic plasticity in the hippocampus and a subsequent decrease in experience-dependent learning (*30*). Therefore, we analyzed the density of bassoon-positive synaptic puncta in single confocal planes along the cell bodies of PNN-coated parvalbumin-positive interneurons. We found no between-group differences in perisomatic excitatory input onto these interneurons (Fig. 4, E and F). Overall, parvalbumin-positive PNN-free neurons received more excitatory input compared to their PNN-coated counterparts, as indicated by increased bassoon-positive puncta (control, 12%; $P = 0.007$). This increase in excitatory input onto parvalbumin-positive PNN-free versus PNN-coated neurons was more pronounced in rats exposed to SDPS (24%; $P = 0.005$; Fig. 4F).

Given the larger number of PNN-coated parvalbumin-positive neurons in SDPS rats versus controls (Fig. 3C), our data suggested that there could be changes in the inhibitory output of parvalbumin-positive interneurons, contributing to the memory deficits observed after SDPS (*31*). To test this, we recorded spontaneous inhibitory postsynaptic currents (sIPSCs) in hippocampal CA1 pyramidal neurons and found that SDPS reduced their frequency (SDPS, 4.56 ± 0.6 Hz; control, 6.67 ± 0.9 Hz; $P = 0.018$; Fig. 4, G and H), without affecting their amplitude ($P = 0.229$; Fig. 4H). Together, our data establish that SDPS increased the number of CSPG-rich PNN-coated parvalbumin-expressing interneurons, which received reduced excitatory perisomatic synaptic input. Furthermore, pyramidal neurons in the hippocampal CA1 subfield of SDPS animals showed reduced inhibitory input.

Extracellular matrix reorganization ameliorates SDPS-induced deficits in hippocampal memory

To assess whether the altered inhibitory tone after SDPS was causally linked to synaptic up-regulation of CSPG expression and the ensuing rise in the number of PNNs, we enzymatically digested CSPGs by intra-hippocampal application of chondroitinase ABC (Fig. 5A). Penicillinase-treated rats were used to control for the stereotactic injection because this enzyme has no endogenous substrate (*32*). We proceeded with cellular, physiological, and behavioral assessments of the effects of chondroitinase ABC at ~2 weeks after administration. This time point was selected to allow for a partial recovery of the extracellular matrix, as reflected by a post-administration increase in the number of PNNs in control animals and an increase in the expression of synaptic CSPGs in SDPS animals (fig. S10).

After the intracranial administration of chondroitinase ABC, the SDPS-induced alteration of the extracellular matrix was normalized (one-way ANOVA; group, $P = 0.008$), as shown by the

1 decreased number of PNNs in the SDPS-chondroitinase group (post hoc SDPS-penicillinase
2 versus control-penicillinase, $P = 0.026$; SDPS-chondroitinase versus control-chondroitinase, $P =$
3 0.281; SDPS-penicillinase versus SDPS-chondroitinase, $P = 0.007$) (Fig. 5, B and C).
4 Chondroitinase ABC treatment decreased the number of PNNs in control rats (fig. S10) (control-
5 chondroitinase versus control-penicillinase, $P = 0.040$).
6 The chondroitinase ABC-induced reorganization of the extracellular matrix normalized the
7 hippocampal inhibitory tone in SDPS rats (one-way ANOVA; group, $P = 0.035$; Fig. 5, D and E).
8 First, we confirmed that SDPS reduced the frequency of sIPSCs onto pyramidal neurons of the
9 hippocampal CA1 region (control-penicillinase, 5.04 ± 0.49 Hz; SDPS-penicillinase, 3.36 ± 0.41
10 Hz; $P = 0.031$). Next, we showed that chondroitinase ABC treatment reversed this effect (SDPS-
11 chondroitinase versus control-chondroitinase, $P = 0.683$; SDPS-chondroitinase versus SDPS-
12 penicillinase, $P = 0.008$), with sIPSC frequency returning to control values (control-
13 chondroitinase, 5.06 ± 0.61 Hz; SDPS-chondroitinase, 5.37 ± 0.58 Hz; $P = 0.979$). No effect on
14 sIPSC amplitude was detected (fig. S11).
15 In independent groups of animals, chondroitinase-induced reorganization of the
16 extracellular matrix rescued the impaired hippocampal plasticity after SDPS (one-way ANOVA;
17 group, $P = 0.040$; Fig. 5, F and G). The robust reduction in LTP maintenance (0.9-fold; SDPS-
18 penicillinase versus control-penicillinase, $P = 0.037$) was absent in chondroitinase ABC-treated
19 rats (SDPS-chondroitinase versus control-chondroitinase, $P = 0.287$; SDPS-chondroitinase versus
20 SDPS-penicillinase, $P = 0.007$). LTP normalization after chondroitinase ABC treatment could be
21 measured for up to 3 weeks after treatment. Given the concordant restoration of LTP and sIPSCs
22 after chondroitinase ABC administration, we next examined whether restoration of the
23 hippocampal network coincided with improved short-term object location memory (Fig. 5, H and

1 I). SDPS-induced deficits on performance on the OPR test were abrogated after chondroitinase
2 ABC administration (two-way ANOVA; group × treatment, *P* = 0.012; post hoc, SDPS-
3 penicillinase versus control-penicillinase, *P* = 0.053; SDPS-chondroitinase versus control-
4 chondroitinase, *P* = 0.044; SDPS-penicillinase versus SDPS-chondroitinase, *P* = 0.004). Notably,
5 whereas object location memory was absent in penicillinase-treated SDPS rats (*P* = 0.477 versus
6 fictive control), chondroitinase-treated SDPS rats displayed intact object location memory (*P* =
7 0.001 versus fictive control), similar to that of penicillinase-treated control rats (*P* = 0.012 versus
8 fictive control). In addition, chondroitinase ABC treatment attenuated the debilitating effects of
9 SDPS on social recognition memory based on the performance in a social recognition test using a
10 juvenile conspecific (fig. S12). Together, these data show that chondroitinase ABC reversed the
11 SDPS-evoked increase in the number of PNN-coated parvalbumin-positive interneurons in the
12 hippocampal CA1 region and restored sIPSC frequency, LTP, and object location and social
13 recognition memory.
14
15 **Discussion**
16 Cognitive impairment associated with MDD has been well characterized (*33*–*35*). This includes
17 deficits in declarative and spatial memory (*36*, *37*), supporting a role for hippocampus-mediated
18 dysfunction and other related (endo)phenotypes, for example, decreased hippocampal volume, in
19 MDD (*38*). However, the molecular mechanisms underlying this association remain to be
20 elucidated. Here, we used a preclinical rat model that induces several long-lasting depressive-like
21 behaviors (*11*, *12*) to investigate the connection between hippocampal pathology and cognitive
22 deficits. Our data indicate a causal relationship between aberrant synaptic CSPG expression,
23 alterations in the number of PNNs, and dysregulation of the hippocampal network that, together,

1 mediate cognitive impairments in our rat model.

2 Collectively, our data highlight the dorsal hippocampus as a principal mediator of cognitive 3 deficits in the SDPS paradigm. At the behavioral level, SDPS impaired short-term object location 4 memory, as assessed by the OPR test (*14*), a task that necessitates recollection of spatial cues and 5 uses the dorsal hippocampus for optimal performance (*18*). SDPS did not affect object recognition 6 in the NOR test, which evaluates the novelty of an object independent of its spatial location and 7 remains intact after loss of most of dorsal hippocampal volume (*18*).

8 At the physiological level, SDPS reduced the plasticity potential of the dorsal 9 hippocampus, as reflected by decreased LTP maintenance, as reported previously (*17*). This 10 synaptic plasticity phenotype correlates with the location memory deficit we observed because it 11 was shown that interference with hippocampal CA1 LTP affects spatial memory performance (*39*, 12 *40*). Important for the predictive validity of our observations was the finding that antidepressant 13 treatment given months after the last exposure to social stress reversed this cognitive phenotype 14 both at the behavioral and physiological level in our SDPS rat model.

15 At the molecular level, analysis of the dorsal hippocampus synaptic proteome in SDPS rats 16 implicated proteins of the extracellular matrix, and in particular, CSPGs, in the observed cognitive 17 impairment and its subsequent rescue by the antidepressant drug imipramine. These changes were 18 most likely occurring in glutamatergic synapses by virtue of the biochemical isolation of the 19 synaptic membrane fraction (*41*). The brevican-rich perisynaptic extracellular matrix (*42*) acts as 20 a diffusion barrier for AMPA receptor lateral mobility, locally altering short-term synaptic 21 plasticity (*43*). Bidirectional alterations in the composition of CSPG-rich extracellular matrix, 22 driven both by genetic (*44–47*) and by pharmacological manipulations (*48–51*), impair 23 hippocampal LTP and hippocampal-mediated memory processes. Thus, it is possible that the

1 robust synaptic up-regulation of CSPGs observed after SDPS affects plasticity at the tetrapartite
2 synapse (*52*), disrupts incoming local and distal excitatory signaling, and thereby impairs
3 hippocampal physiology and memory formation and recall. Indeed, changes in matrix
4 metalloproteinase activity, which regulate extracellular matrix proteolysis, have been reported to
5 drive stress-induced CA1-mediated cognitive deficits (*53*).

6 At the cellular level, SDPS-induced effects on PNNs were linked to interneurons of the
7 CA1 stratum pyramidale that expressed parvalbumin. In particular, we showed that SDPS induced
8 an increase in the number of parvalbumin-expressing interneurons coated by PNNs. This was in
9 parallel with increased expression of parvalbumin selectively in PNN-coated interneurons that
10 received less excitatory perisomatic synaptic input compared to their PNN-free counterparts. PNN
11 organization is critical for the intrinsic structural and functional properties of parvalbumin-
12 expressing neurons (*54–56*), including regulation of their excitability (*28*). Notably, the presence
13 of PNNs has been reported to correlate directly with the expression of parvalbumin (*29*, *57*), which
14 is a hallmark of cellular activity (*30*).

15 Our data argue that SDPS-induced adaptations in PNN-coated parvalbumin-positive
16 neurons of the hippocampus CA1 region, together with the observed increase in perisynaptic
17 extracellular matrix, may elicit a reduction in the inhibitory output of parvalbumin-positive
18 interneurons, leading to decreased sIPSC frequency in hippocampal CA1 principal neurons.
19 Supporting this notion, after chronic mild stress, an antidepressant-reversible reduction in sIPSC
20 frequency has been associated with decreased GABA release probability in the hippocampus
21 dentate gyrus (*58*). Likewise, an imipramine-induced increase in sIPSC frequency was
22 accompanied by altered GABA presynaptic release in the hippocampus CA1 region (*59*).

23 Although sIPSCs represent the combined diverse inhibitory inputs that characterize the

1 hippocampal network (*60*), we hypothesized that the observed effect of decreased inhibitory input
2 is driven by reduced parvalbumin-dependent perisomatic inhibition, which is the predominant
3 inhibitory input onto hippocampus CA1 pyramidal cells (*61*, *62*). We show that in rats exposed to
4 the SDPS paradigm and treated with chondroitinase ABC, there was a restoration of the number
5 of PNN-coated parvalbumin-positive interneurons and a rescue of the sIPSC phenotype.
6 Chondroitinase-mediated PNN removal has been reported to increase the excitability of
7 parvalbumin-positive neurons in vitro (*28*), indicating that an aberrant increase in extracellular
8 matrix could lead to a reduction in interneuron excitability and a subsequent decrease in sIPSC
9 frequency. Our data showing reduced excitatory puncta in PNN-coated parvalbumin-positive
10 neurons support this hypothesis.

11 Parvalbumin-positive neurons are essential for proper functioning of the hippocampal
12 network through their direct effects on hippocampal CA1 principal neurons (*63*, *64*) and
13 subsequent modulation of hippocampal gamma oscillations (*65*, *66*). We demonstrated that
14 restoration of the number of PNN-coated parvalbumin-positive neurons by intrahippocampal
15 administration of chondroitinase ABC coincided with improved hippocampal inhibitory tone
16 (sIPSC frequency) and plasticity (LTP maintenance). We propose that there may be a common
17 extracellular matrix–associated molecular mechanism that drives hippocampal pathology after
18 SDPS. In line with this, transgenic mice deficient in the *TnR* gene, which show reduced perisomatic
19 inhibition, display a metaplastic increase in LTP induction threshold (*67*), indicating
20 interdependence between extracellular matrix, inhibitory transmission, and plasticity in the
21 hippocampus. Furthermore, chondroitinase ABC administration rescued SDPS-induced cognitive
22 deficits on object location, suggesting that impaired hippocampus-mediated memory function is
23 due to extracellular matrix changes at both the perisynaptic (that is, CSPGs) and the pericellular

(that is, PNN) levels.

An attractive hypothesis is that the presence of PNNs (*29*, *32*), similar to increased expression of parvalbumin (*30*), marks the maturation of parvalbumin-positive interneurons. Thereafter, these cells participate in a network configuration that is characterized by low plasticity used to maintain already established behavioral patterns (*68*, *69*). In our rat model that shows a sustained depressive-like state, elevated synaptic CSPG expression, and the increased number of PNN-coated parvalbumin-positive interneurons in the hippocampus CA1 region may have contributed to reduced hippocampal plasticity, promoted the embedding of maladaptive memories, and hindered the (re)consolidation of (updated) information, as shown previously (*70*, *71*). The extracellular matrix reorganization after either chronic imipramine treatment or a single chondroitinase ABC treatment could act to boost hippocampal plasticity and subsequently memory function in rats exposed to the SDPS paradigm. Supporting this notion, chronic fluoxetine treatment in mice was reported to reduce the number of PNN-coated parvalbumin-positive neurons in hippocampal CA1 and in the amygdala, rendering parvalbumin-positive neurons in a state of dematuration (*70*). This effect was associated with a reactivation of juvenile plasticity that facilitated memory processes, including memory overwrite and incorporation of updated information (*70*, *71*).

There are a number of limitations to our study. Although we found evidence for extracellular matrix-associated alterations in the cognitive component associated with depressive-like behavior, what drives these changes remains to be understood. Future studies will need to examine the role of cell-type specific contributions to synthesis, release, and degradation of extracellular matrix proteins in the SDPS model. Likewise, it would be useful to examine whether extracellular matrix–related changes are seen in different brain areas (for example, cortical areas)

known to be associated with cognitive deficits in depression (*72*). Moreover, it will be of interest to investigate whether the antidepressant effects of chondroitinase ABC on cognitive behavior last beyond 3 weeks and, if so, how the interplay between extracellular matrix production and breakdown is regulated in the long term.

Preclinical data in animal models, such as our SDPS rat model showing several depressive-like behaviors, need to be interpreted with caution. Our data would be strengthened by clinical evidence of extracellular matrix-related changes in postmortem brain tissue from MDD patients, as has been shown for schizophrenia (*73*). Moreover, although SDPS affects both object place and social recognition memory (*11*) and both are ameliorated by chondroitinase ABC treatment, future studies will need to investigate other type of cognitive behaviors.

Our data indicate that components of the extracellular matrix contribute to reduced plasticity potential and impaired memory processes in the SDPS rat model. Our study suggests that translational strategies aimed at restoring altered extracellular matrix organization, PNN integrity, or related inhibitory network function (*74*) deserve further exploration as potential targets for alleviating cognitive deficits in MDD.

Materials and Methods

Study design

The present study consists of a series of experiments using multiple molecular (biochemical assays and proteomics), cellular (immuno-histochemistry and electrophysiology), and behavioral techniques to examine sustained depressive-like behavior in the SDPS rat model. Independent groups of animals were used for each technique and to cross-validate results [for example, isobaric tags for relative and absolute quantitation (iTRAQ)–based proteomics versus proteomic analysis

using immunoblots] or to investigate treatment effects (for example, the effect of chondroitinase ABC treatment on sIPSCs). Groups were randomly assigned, except for intervention experiments (Fig. 5), in which groups were balanced using baseline OPR test results. When applicable (Fig. 5, F to I), experiments were carried out using independent batches of animals yet combining experiments with low impact and carryover to adhere to the 3-R principle of ethical use of experimental animals. No between-batch differences were observed. In all experiments, researchers were blinded to the group or treatment protocol when measurements were being taken and upon initial analysis of between group effects.

Animals

Male Wistar rats (Harlan) aged 6 to 8 weeks were habituated after arrival to housing, handling, and reversed day/night cycle (2 weeks). Rats were exposed to the SDPS paradigm (*13*), starting with five single daily exposures to social defeat stress. From the first defeat episode onward, SDPS rats (≥9 weeks old) were single-housed, deprived from standard home-cage enrichment. Control rats were pair-housed and daily handled and/or exposed to an empty social defeat apparatus during the defeat exposure of the SDPS group. Individually housed controls were isolated for a period of 2 to 3 months, devoid of defeat. Whenever applicable, the antidepressant imipramine (20 mg/kg per 0.5 mL of water; Sigma-Aldrich) was orally (gavage or via water bottle) administrated during the last 3 weeks of the social isolation period. All behavioral, electrophysiological, and molecular analyses were performed at the end of treatment/after intervention in independent groups of rats 2 to 3 months after the last defeat, unless stated otherwise. All experiments were approved by the Animal Users Care Committee of the VU University Amsterdam and were performed in accordance with the relevant guidelines and regulations.

Cognitive assessment

Object place recognition (OPR) task

Hippocampal-dependent short-term object location memory was determined with the OPR test (*14*) using a 15-min retention interval. Discrimination between spatial locations of objects was used as measurement for spatial memory [exploration index = time spent in active zone (novel location)/total exploration time (novel + familiar location)] in a 4-min test. The configuration of the object's novel place was counterbalanced such that on each trial, a different corner was used as a familiar and novel location. Objects were randomly assigned between groups to avoid the development of preference.

Novel object recognition (NOR) task

Short-term recognition memory was determined with the NOR test using a 15-min retention interval. Discrimination between objects was estimated on the basis of preference for the novel object [exploration index = time spent in active zone (novel object)/total exploration time (novel + familiar object)]. Objects were randomly assigned between groups to avoid development of preference.

Chondroitinase ABC administration

SDPS and control rats received a single infusion of 0.03 U per side of chondroitinase ABC (C3667, Sigma-Aldrich) or penicillinase (P0389, Sigma-Aldrich) in a 0.5-µL volume in the dorsal hippocampus (bregma: −3.8 anterior-posterior, ±2.1 medial-lateral, and −2.9 dorso-ventral) >2 months after the last social defeat trial. Chondroitinase ABC effects in SDPS (OPR, PNNs, and e-phys) were assessed at ≥2 weeks after administration using two batches of animals. The first batch was used for OPR test and LTP measurements. All animals received chondroitinase ABC or

penicillinase treatment, and OPR memory was tested at 12 days after the operations. Thereafter, LTP was analyzed between 16 and 24 days after chondroitinase ABC application (see Fig. 5A). The second batch was used for PNN quantification and for sIPSC recordings. Similar to the first batch, all animals received chondroitinase ABC or penicillinase and were subjected to the OPR task at 10 to 12 days after administration. Thereafter, all animals were decapitated, and sIPSC recordings were obtained 24 to 96 hours after the OPR test. For PNNs, animals were perfused 24 to 48 hours after the OPR test.

Immunohistochemistry

Sections from SDPS and control (Figs. 3 to 5 and figs. S7 to S9) rats were labeled overnight with mouse anti–chondroitin sulfate proteoglycan [1:1000; clone Cat-301 (*54*) MAB5284, Chemicon/ Millipore], rabbit anti-parvalbumin (1:1000; PV 28, Swant Inc.), mouse anti-aggrecan (1:1000; AB1031, Millipore), and goat anti-bassoon (1:1000; Novus Biological) and subsequently imaged using a Leica DM5000 B (PNNs and PV+ cell numbers and intensity) or a Carl Zeiss Axiovert 200M (Bs+ puncta) microscope. For quantification, images taken using the Leica Application Suite software (2.7.2 9586 Advanced Fluorescence, Leica Microsystems) or LSM 510 software (version 4.2) were analyzed by automated in-house FIJI (*75*) scripts. Parvalbumin intensity (Fig. 4, E and F) was calculated on the basis of average intensity values measured in controls. Parvalbumin-positive interneurons were subdivided into fractions according to the intensity in controls, as follows: <60%, low; 61 to 85%, intermediate low; 86 to 110%, intermediate high; >111%, high. The number of bassoon-positive synaptic puncta per cell was calculated by dividing the area of the soma (arbitrary units) due to the variation in size in parvalbumin-positive cell bodies. Independent sets of animals were used for quantification of PNNs in the three hippocampus

subfields (CA1, CA2/3, and DG), at CA1 layers, and after chondroitinase ABC administration, as well as for quantification of bassoon-positive puncta.

Immunoblotting

Total homogenate (fig. S5) and synaptic membranes of the dorsal hippocampus (Fig. 2) were isolated from independent groups of animals. For CSPGs immunoblotting, samples were treated [chondroitinase ABC, 90 min at 37 °C using 0.002 U/μL in NaAc (pH 8.0)] before SDS-gel separation. Samples (10 μg) were lysed in Laemmli lysis buffer, separated by electrophoresis on gradient precast gels (4 to 20%; Criterion TGX stain-free, Bio-Rad), and blotted to polyvinylidene difluoride membrane (Bio-Rad). Primary antibodies used were rabbit anti-aggrecan (1:700; AB1031, Millipore), guinea pig anti-brevican (1:2000; provided by C. I. Seidenbecher, Magdeburg), mouse anti-neurocan (1:1000; N0913, Alpha Diagnostics), mouse anti-phosphacan (1:1000; 3F8, Developmental Studies Hybridoma Bank), mouse anti-versican (1:1000; 75-324, NeuroMab), mouse anti–tenascin-R (1:2000; mTN-R2, Acris Antibodies), and rabbit anti-HPLN1 (1:1000; ab98038, Abcam). After incubation with horseradish peroxidase–conjugated secondary antibody (1:10,000; Dako) and visualization with Femto Chemiluminescent Substrate (Thermo Fisher Scientific), blots were scanned using the LI-COR Odyssey Fc and analyzed with Image Studio (LI-COR). Total protein was visualized using trichloroethanol staining, scanned using a Gel Doc EZ imager (Bio-Rad), and analyzed with Image Lab (Bio-Rad) to correct for input differences per sample because this is a reliable method that is not dependent on a single protein for normalization (*76*). Water- and imipramine-treated samples were run on separate gels. All samples (SDPS-H2O, Con-imipramine, and SDPS-imipramine) were run adjacent to Con-H2O samples; thus, all values are expressed as fold change from control. In Fig. 2, the mean of two

SDPS samples was each time quantified versus their adjacent control sample.

Electrophysiology

For details on whole-cell patch-clamp recordings (IPSC measurement) and LTP measurements, see Supplementary Materials and Methods.

Statistical analysis

Memory retention in the OPR test, NOR test (Figs. 1 and 5), and social recognition memory task, as well as approach behavior in the social approach avoidance task (fig. S12), was statistically tested by comparing the data (Student's *t* test) to a fictive control, as reported previously (*16*). The ratio of exploration or interaction, based on the time spent exploring an object or interacting with a social target, was 0.5 for the fictive control, representing task performance at chance levels while retaining a similar distribution and within-sample variation as the original data. This stringent approach gives a more realistic comparison with higher statistical power than performing a single-sample *t* test (*16*). For the iTRAQ-based proteomics, multiple comparisons correction was carried out using the power law global error model (PLGEM) (*20*) or using the WebGestalt GO enrichment analysis. For all other data, statistical analysis was performed using SPSS21.0.

The effects of SDPS and of treatments were assessed with one-way or two-way ANOVA, followed by Fisher's least significant difference (LSD) post hoc analyses. Mann-Whitney nonparametric test was used in cases of non-normal data distribution. Paired sample *t* tests were used for within-group comparisons. Testing was two-sided, unless the initial experiment (proteomics to immunoblot, CA1 to CA1 layers, and OPR test performance at the intervention experiment) directed follow-up studies. All results are expressed as group mean ± SEM. Statistical

outliers were excluded only in the case of a value exceeding 2× standard deviation of the group average on multiple parameters, leading to the exclusion of the following data: immuno-blotting, $n = 1$ for control-H2O; electrophysiology (sIPSC frequency), $n = 1$ for the SDPS group and $n = 1$ for the SDPS-penicillinase group. All statistical tests performed are summarized in table S2.

1 **References**

2 1. R. H. Belmaker, G. Agam, Major depressive disorder. *N Engl J Med* **358**, 55-68 (2008).
3 2. J. W. Murrough, B. Iacoviello, A. Neumeister, D. S. Charney, D. V. Iosifescu, Cognitive dysfunction in
4 depression: neurocircuitry and new therapeutic strategies. *Neurobiol Learn Mem* **96**, 553-563 (2011).
5 3. B. T. Baune, R. Miller, J. McAfoose, M. Johnson, F. Quirk, D. Mitchell, The role of cognitive impairment
6 in general functioning in major depression. *Psychiatry research* **176**, 183-189 (2010).
7 4. J. D. Bremner, M. Narayan, E. R. Anderson, L. H. Staib, H. L. Miller, D. S. Charney, Hippocampal volume
8 reduction in major depression. *The American journal of psychiatry* **157**, 115-118 (2000).
9 5. S. L. Fairhall, S. Sharma, J. Magnusson, B. Murphy, Memory related dysregulation of hippocampal
10 function in major depressive disorder. *Biological psychology* **85**, 499-503 (2010).
11 6. C. Hammen, Stress and depression. *Annu Rev Clin Psychol* **1**, 293-319 (2005).
12 7. K. Wingenfeld, O. T. Wolf, Stress, memory, and the hippocampus. *Frontiers of neurology and*
13 *neuroscience* **34**, 109-120 (2014).
14 8. J. J. Kim, D. M. Diamond, The stressed hippocampus, synaptic plasticity and lost memories. *Nature*
15 *reviews. Neuroscience* **3**, 453-462 (2002).
16 9. C. Pittenger, R. S. Duman, Stress, depression, and neuroplasticity: a convergence of mechanisms.
17 *Neuropsychopharmacology* **33**, 88-109 (2008).
18 10. J. C. Von Frijtag, L. G. Reijmers, J. E. Van der Harst, I. E. Leus, R. Van den Bos, B. M. Spruijt, Defeat
19 followed by individual housing results in long-term impaired reward- and cognition-related behaviours in
20 rats. *Behav Brain Res* **117**, 137-146 (2000).
21 11. D. Riga, L. J. Schmitz, J. E. van der Harst, Y. van Mourik, W. J. Hoogendijk, A. B. Smit, T. J. De Vries, S.
22 Spijker, A sustained depressive state promotes a guanfacine reversible susceptibility to alcohol seeking in
23 rats. *Neuropsychopharmacology* **39**, 1115-1124 (2014).
24 12. D. Riga, J. T. Theijs, T. J. De Vries, A. B. Smit, S. Spijker, Social defeat-induced anhedonia: effects on
25 operant sucrose-seeking behavior. *Front Behav Neurosci* **9**, 195 (2015).
26 13. P. Van Bokhoven, C. A. Oomen, W. J. Hoogendijk, A. B. Smit, P. J. Lucassen, S. Spijker, Reduction in
27 hippocampal neurogenesis after social defeat is long-lasting and responsive to late antidepressant treatment.
28 *Eur J Neurosci* **33**, 1833-1840 (2011).
29 14. E. Dere, J. P. Huston, M. A. De Souza Silva, The pharmacology, neuroanatomy and neurogenetics of one-
30 trial object recognition in rodents. *Neurosci Biobehav Rev* **31**, 673-704 (2007).
31 15. M. Antunes, G. Biala, The novel object recognition memory: neurobiology, test procedure, and its
32 modifications. *Cogn Process* **13**, 93-110 (2012).
33 16. S. Akkerman, J. Prickaerts, H. W. Steinbusch, A. Blokland, Object recognition testing: statistical
34 considerations. *Behavioural brain research* **232**, 317-322 (2012).
35 17. J. C. Von Frijtag, A. Kamal, L. G. Reijmers, L. H. Schrama, R. van den Bos, B. M. Spruijt, Chronic
36 imipramine treatment partially reverses the long-term changes of hippocampal synaptic plasticity in
37 socially stressed rats. *Neurosci Lett* **309**, 153-156 (2001).
38 18. N. J. Broadbent, L. R. Squire, R. E. Clark, Spatial memory, recognition memory, and the hippocampus.
39 *Proceedings of the National Academy of Sciences of the United States of America* **101**, 14515-14520
40 (2004).
41 19. D. M. Bannerman, R. Sprengel, D. J. Sanderson, S. B. McHugh, J. N. Rawlins, H. Monyer, P. H. Seeburg,
42 Hippocampal synaptic plasticity, spatial memory and anxiety. *Nature reviews. Neuroscience* **15**, 181-192
43 (2014).
44 20. N. Pavelka, M. Pelizzola, C. Vizzardelli, M. Capozzoli, A. Splendiani, F. Granucci, P. Ricciardi-
45 Castagnoli, A power law global error model for the identification of differentially expressed genes in
46 microarray data. *BMC Bioinformatics* **5**, 203 (2004).
47 21. B. Zhang, S. Kirov, J. Snoddy, WebGestalt: an integrated system for exploring gene sets in various
48 biological contexts. *Nucleic acids research* **33**, W741-748 (2005).
49 22. A. Dityatev, M. Schachner, Extracellular matrix molecules and synaptic plasticity. *Nat Rev Neurosci* **4**,
50 456-468 (2003).
51 23. A. Faissner, M. Pyka, M. Geissler, T. Sobik, R. Frischknecht, E. D. Gundelfinger, C. Seidenbecher,
52 Contributions of astrocytes to synapse formation and maturation - Potential functions of the perisynaptic
53 extracellular matrix. *Brain Res Rev* **63**, 26-38 (2010).
54 24. A. Dityatev, D. A. Rusakov, Molecular signals of plasticity at the tetrapartite synapse. *Curr Opin Neurobiol*
55 **21**, 353-359 (2011).

1 25. W. Hartig, K. Brauer, V. Bigl, G. Bruckner, Chondroitin sulfate proteoglycan-immunoreactivity of lectin-
2 labeled perineuronal nets around parvalbumin-containing neurons. *Brain research* **635**, 307-311 (1994).
3 26. S. S. Deepa, D. Carulli, C. Galtrey, K. Rhodes, J. Fukuda, T. Mikami, K. Sugahara, J. W. Fawcett,
4 Composition of perineuronal net extracellular matrix in rat brain: a different disaccharide composition for
5 the net-associated proteoglycans. *The Journal of biological chemistry* **281**, 17789-17800 (2006).
6 27. C. Romberg, S. Yang, R. Melani, M. R. Andrews, A. E. Horner, M. G. Spillantini, T. J. Bussey, J. W.
7 Fawcett, T. Pizzorusso, L. M. Saksida, Depletion of perineuronal nets enhances recognition memory and
8 long-term depression in the perirhinal cortex. *J Neurosci* **33**, 7057-7065 (2013).
9 28. A. Dityatev, G. Bruckner, G. Dityateva, J. Grosche, R. Kleene, M. Schachner, Activity-dependent
10 formation and functions of chondroitin sulfate-rich extracellular matrix of perineuronal nets. *Dev Neurobiol*
11 **67**, 570-588 (2007).
12 29. J. Yamada, T. Ohgomori, S. Jinno, Perineuronal nets affect parvalbumin expression in GABAergic neurons
13 of the mouse hippocampus. *Eur J Neurosci* **41**, 368-378 (2015).
14 30. F. Donato, S. B. Rompani, P. Caroni, Parvalbumin-expressing basket-cell network plasticity induced by
15 experience regulates adult learning. *Nature* **504**, 272-276 (2013).
16 31. A. J. Murray, J. F. Sauer, G. Riedel, C. McClure, L. Ansel, L. Cheyne, M. Bartos, W. Wisden, P. Wulff,
17 Parvalbumin-positive CA1 interneurons are required for spatial working but not for reference memory.
18 *Nature neuroscience* **14**, 297-299 (2011).
19 32. T. Pizzorusso, P. Medini, N. Berardi, S. Chierzi, J. W. Fawcett, L. Maffei, Reactivation of ocular
20 dominance plasticity in the adult visual cortex. *Science* **298**, 1248-1251 (2002).
21 33. A. Hammar, G. Ardal, Cognitive functioning in major depression--a summary. *Frontiers in human*
22 *neuroscience* **3**, 26 (2009).
23 34. D. Marazziti, G. Consoli, M. Picchetti, M. Carlini, L. Faravelli, Cognitive impairment in major depression.
24 *European journal of pharmacology* **626**, 83-86 (2010).
25 35. P. L. Rock, J. P. Roiser, W. J. Riedel, A. D. Blackwell, Cognitive impairment in depression: a systematic
26 review and meta-analysis. *Psychological medicine* **44**, 2029-2040 (2014).
27 36. N. F. Gould, M. K. Holmes, B. D. Fantie, D. A. Luckenbaugh, D. S. Pine, T. D. Gould, N. Burgess, H. K.
28 Manji, C. A. Zarate, Jr., Performance on a virtual reality spatial memory navigation task in depressed
29 patients. *The American journal of psychiatry* **164**, 516-519 (2007).
30 37. M. Dresler, M. Kluge, L. Genzel, P. Schussler, A. Steiger, Impaired off-line memory consolidation in
31 depression. *European neuropsychopharmacology : the journal of the European College of*
32 *Neuropsychopharmacology* **20**, 553-561 (2010).
33 38. S. Campbell, G. Macqueen, The role of the hippocampus in the pathophysiology of major depression.
34 *Journal of psychiatry & neuroscience : JPN* **29**, 417-426 (2004).
35 39. C. Pittenger, Y. Y. Huang, R. F. Paletzki, R. Bourtchouladze, H. Scanlin, S. Vronskaya, E. R. Kandel,
36 Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts
37 hippocampus-dependent spatial memory. *Neuron* **34**, 447-462 (2002).
38 40. T. Okada, N. Yamada, K. Tsuzuki, H. P. Horikawa, K. Tanaka, S. Ozawa, Long-term potentiation in the
39 hippocampal CA1 area and dentate gyrus plays different roles in spatial learning. *Eur J Neurosci* **17**, 341-
40 349 (2003).
41 41. K. W. Li, M. P. Hornshaw, R. C. Van der Schors, R. Watson, S. Tate, B. Casetta, C. R. Jimenez, Y.
42 Gouwenberg, E. D. Gundelfinger, K. H. Smalla, A. B. Smit, Proteomics Analysis of Rat Brain Postsynaptic
43 Density - Implications of the diverse protein functional groups for the integration of synaptic physiology
44 *The Journal of biological chemistry* **279**, 987-1002 (2004).
45 42. A. Dityatev, C. I. Seidenbecher, M. Schachner, Compartmentalization from the outside: the extracellular
46 matrix and functional microdomains in the brain. *Trends Neurosci* **33**, 503-512 (2010).
47 43. R. Frischknecht, M. Heine, D. Perrais, C. I. Seidenbecher, D. Choquet, E. D. Gundelfinger, Brain
48 extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. *Nat*
49 *Neurosci* **12**, 897-904 (2009).
50 44. A. K. Saghatelyan, A. Dityatev, S. Schmidt, T. Schuster, U. Bartsch, M. Schachner, Reduced perisomatic
51 inhibition, increased excitatory transmission, and impaired long-term potentiation in mice deficient for the
52 extracellular matrix glycoprotein tenascin-R. *Mol Cell Neurosci* **17**, 226-240 (2001).
53 45. D. Carulli, T. Pizzorusso, J. C. Kwok, E. Putignano, A. Poli, S. Forostyak, M. R. Andrews, S. S. Deepa, T.
54 T. Glant, J. W. Fawcett, Animals lacking link protein have attenuated perineuronal nets and persistent
55 plasticity. *Brain : a journal of neurology* **133**, 2331-2347 (2010).

46. M. Geissler, C. Gottschling, A. Aguado, U. Rauch, C. H. Wetzel, H. Hatt, A. Faissner, Primary hippocampal neurons, which lack four crucial extracellular matrix molecules, display abnormalities of synaptic structure and function and severe deficits in perineuronal net formation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **33**, 7742-7755 (2013).
47. B. R. Lubbers, M. R. Matos, A. Horn, E. Visser, R. C. Van der Loo, Y. Gouwenberg, G. F. Meerhoff, R. Frischknecht, C. I. Seidenbecher, A. B. Smit, S. Spijker, M. C. van den Oever, The Extracellular Matrix Protein Brevican Limits Time-Dependent Enhancement of Cocaine Conditioned Place Preference. *Neuropsychopharmacology* **41**, 1907-1916 (2016).
48. O. Bukalo, M. Schachner, A. Dityatev, Modification of extracellular matrix by enzymatic removal of chondroitin sulfate and by lack of tenascin-R differentially affects several forms of synaptic plasticity in the hippocampus. *Neuroscience* **104**, 359-369 (2001).
49. S. E. Meighan, P. C. Meighan, P. Choudhury, C. J. Davis, M. L. Olson, P. A. Zornes, J. W. Wright, J. W. Harding, Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. *J Neurochem* **96**, 1227-1241 (2006).
50. V. Nagy, O. Bozdagi, G. W. Huntley, The extracellular protease matrix metalloproteinase-9 is activated by inhibitory avoidance learning and required for long-term memory. *Learn Mem* **14**, 655-664 (2007).
51. M. J. Hylin, S. A. Orsi, A. N. Moore, P. K. Dash, Disruption of the perineuronal net in the hippocampus or medial prefrontal cortex impairs fear conditioning. *Learn Mem* **20**, 267-273 (2013).
52. R. Frischknecht, E. D. Gundelfinger, The brain's extracellular matrix and its role in synaptic plasticity. *Adv Exp Med Biol* **970**, 153-171 (2012).
53. M. A. van der Kooij, M. Fantin, E. Rejmak, J. Grosse, O. Zanoletti, C. Fournier, K. Ganguly, K. Kalita, L. Kaczmarek, C. Sandi, Role for MMP-9 in stress-induced downregulation of nectin-3 in hippocampal CA1 and associated behavioural alterations. *Nat Commun* **5**, 4995 (2014).
54. K. Schuppel, K. Brauer, W. Hartig, J. Grosche, B. Earley, B. E. Leonard, G. Bruckner, Perineuronal nets of extracellular matrix around hippocampal interneurons resist destruction by activated microglia in trimethyltin-treated rats. *Brain Res* **958**, 448-453 (2002).
55. J. H. Cabungcal, P. Steullet, H. Morishita, R. Kraftsik, M. Cuenod, T. K. Hensch, K. Q. Do, Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proc Natl Acad Sci U S A* **110**, 9130-9135 (2013).
56. R. Guirado, M. Perez-Rando, D. Sanchez-Matarredona, E. Castren, J. Nacher, Chronic fluoxetine treatment alters the structure, connectivity and plasticity of cortical interneurons. *Int J Neuropsychopharmacol* **17**, 1635-1646 (2014).
57. K. Ohira, R. Takeuchi, T. Iwanaga, T. Miyakawa, Chronic fluoxetine treatment reduces parvalbumin expression and perineuronal nets in gamma-aminobutyric acidergic interneurons of the frontal cortex in adult mice. *Mol Brain* **6**, 43 (2013).
58. M. M. Holm, J. L. Nieto-Gonzalez, I. Vardya, K. Henningsen, M. N. Jayatissa, O. Wiborg, K. Jensen, Hippocampal GABAergic dysfunction in a rat chronic mild stress model of depression. *Hippocampus* **21**, 422-433 (2011).
59. P. Mendez, A. Pazienti, G. Szabo, A. Bacci, Direct alteration of a specific inhibitory circuit of the hippocampus by antidepressants. *J Neurosci* **32**, 16616-16628 (2012).
60. T. Klausberger, GABAergic interneurons targeting dendrites of pyramidal cells in the CA1 area of the hippocampus. *The European journal of neuroscience* **30**, 947-957 (2009).
61. T. F. Freund, I. Katona, Perisomatic inhibition. *Neuron* **56**, 33-42 (2007).
62. M. Megias, Z. Emri, T. F. Freund, A. I. Gulyas, Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience* **102**, 527-540 (2001).
63. T. Klausberger, P. Somogyi, Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* **321**, 53-57 (2008).
64. E. Campanac, C. Gasselin, A. Baude, S. Rama, N. Ankri, D. Debanne, Enhanced intrinsic excitability in basket cells maintains excitatory-inhibitory balance in hippocampal circuits. *Neuron* **77**, 712-722 (2013).
65. B. Lasztoczi, T. Klausberger, Layer-specific GABAergic control of distinct gamma oscillations in the CA1 hippocampus. *Neuron* **81**, 1126-1139 (2014).
66. E. Stark, L. Roux, R. Eichler, Y. Senzai, S. Royer, G. Buzsaki, Pyramidal cell-interneuron interactions underlie hippocampal ripple oscillations. *Neuron* **83**, 467-480 (2014).
67. O. Bukalo, M. Schachner, A. Dityatev, Hippocampal metaplasticity induced by deficiency in the extracellular matrix glycoprotein tenascin-R. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 6019-6028 (2007).

68. T. S. Balmer, V. M. Carels, J. L. Frisch, T. A. Nick, Modulation of perineuronal nets and parvalbumin with developmental song learning. *J Neurosci* **29**, 12878-12885 (2009).
69. S. Karunakaran, A. Chowdhury, F. Donato, C. Quairiaux, C. M. Michel, P. Caroni, PV plasticity sustained through D1/5 dopamine signaling required for long-term memory consolidation. *Nat Neurosci* **19**, 454-464 (2016).
70. N. N. Karpova, A. Pickenhagen, J. Lindholm, E. Tiraboschi, N. Kulesskaya, A. Agustsdottir, H. Antila, D. Popova, Y. Akamine, A. Bahi, R. Sullivan, R. Hen, L. J. Drew, E. Castren, Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science* **334**, 1731-1734 (2011).
71. N. Gogolla, P. Caroni, A. Luthi, C. Herry, Perineuronal nets protect fear memories from erasure. *Science* **325**, 1258-1261 (2009).
72. A. Etkin, A. Gyurak, R. O'Hara, A neurobiological approach to the cognitive deficits of psychiatric disorders. *Dialogues Clin Neurosci* **15**, 419-429 (2013).
73. S. Berretta, H. Pantazopoulos, M. Markota, C. Brown, E. T. Batzianouli, Losing the sugar coating: Potential impact of perineuronal net abnormalities on interneurons in schizophrenia. *Schizophrenia research*, (2015).
74. B. Luscher, Q. Shen, N. Sahir, The GABAergic deficit hypothesis of major depressive disorder. *Molecular psychiatry* **16**, 383-406 (2011).
75. J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, A. Cardona, Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**, 676-682 (2012).
76. M. C. Van den Oever, B. R. Lubbers, N. A. Goriounova, K. W. Li, R. C. Van der Schors, M. Loos, D. Riga, J. Wiskerke, R. Binnekade, M. Stegeman, A. N. Schoffelmeer, H. D. Mansvelder, A. B. Smit, T. J. De Vries, S. Spijker, Extracellular matrix plasticity and GABAergic inhibition of prefrontal cortex pyramidal cells facilitates relapse to heroin seeking. *Neuropsychopharmacology* **35**, 2120-2133 (2010).
77. N. J. Broadbent, S. Gaskin, L. R. Squire, R. E. Clark, Object recognition memory and the rodent hippocampus. Learn Mem 17, 5-11 (2010).
78 J. T. Ting, T. L. Daigle, Q. Chen, G. Feng, Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. *Methods Mol Biol* **1183**, 221-242 (2014).
79. K. Shimono, M. Baudry, L. Ho, M. Taketani, G. Lynch, Long-term recording of LTP in cultured hippocampal slices. *Neural Plast* **9**, 249-254 (2002).
80. M. C. Van den Oever, N. A. Goriounova, K. W. Li, R. C. Van der Schors, R. Binnekade, A. N. Schoffelmeer, H. D. Mansvelder, A. B. Smit, S. Spijker, T. J. De Vries, Prefrontal cortex AMPA receptor plasticity is crucial for cue-induced relapse to heroin-seeking. *Nature neuroscience* **11**, 1053-1058 (2008).
81. D. S. Counotte, N. A. Goriounova, K. W. Li, M. Loos, R. C. van der Schors, D. Schetters, A. N. Schoffelmeer, A. B. Smit, H. D. Mansvelder, T. Pattij, S. Spijker, Lasting synaptic changes underlie attention deficits caused by nicotine exposure during adolescence. *Nature neuroscience* **14**, 417-419 (2011).
82. K. W. Li, M. P. Hornshaw, R. C. Van der Schors, R. Watson, S. Tate, B. Casetta, C. R. Jimenez, Y. Gouwenberg, E. D. Gundelfinger, K. H. Smalla, A. B. Smit, Proteomics Analysis of Rat Brain Postsynaptic Density - Implications of the diverse protein functional groups for the integration of synaptic physiology The Journal of biological chemistry 279, 987-1002 (2004).
83. K. Meyer-Arendt, W. M. Old, S. Houel, K. Renganathan, B. Eichelberger, K. A. Resing, N. G. Ahn, IsoformResolver: A peptide-centric algorithm for protein inference. Journal of proteome research 10, 3060-3075 (2011).
84. L. Reiter, M. Claassen, S. P. Schrimpf, M. Jovanovic, A. Schmidt, J. M. Buhmann, M. O. Hengartner, R. Aebersold, Protein identification false discovery rates for very large proteomics data sets generated by tandem mass spectrometry. Molecular & cellular proteomics : MCP 8, 2405-2417 (2009).
85. N. A. Karp, W. Huber, P. G. Sadowski, P. D. Charles, S. V. Hester, K. S. Lilley, Addressing accuracy and precision issues in iTRAQ quantitation. Molecular & cellular proteomics : MCP 9, 1885-1897 (2010).
86. C. Lander, P. Kind, M. Maleski, S. Hockfield, A family of activity-dependent neuronal cell-surface chondroitin sulfate proteoglycans in cat visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 17, 1928-1939 (1997).
87. J. M. Ajmo, A. K. Eakin, M. G. Hamel, P. E. Gottschall, Discordant localization of WFA reactivity and brevican/ADAMTS-derived fragment in rodent brain. BMC neuroscience 9, 14 (2008).
88. P. Garrido, M. de Blas, A. Del Arco, G. Segovia, F. Mora, Aging increases basal but not stress-induced levels of corticosterone in the brain of the awake rat. Neurobiol Aging 33, 375-382 (2012).

89. E. H. Umeoka, S. B. Garcia, J. Antunes-Rodrigues, L. L. Elias, N. Garcia-Cairasco, Functional characterization of the hypothalamic-pituitary-adrenal axis of the Wistar Audiogenic Rat (WAR) strain. Brain Res 1381, 141-7 (2011).
90. G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates - The New Coronal Set. (Elsevier Academic Press, Burlington, MA, ed. 5th, 2005).
91. F. L. Hitti, S. A. Siegelbaum, The hippocampal CA2 region is essential for social memory. Nature 508, 88-92 (2014).

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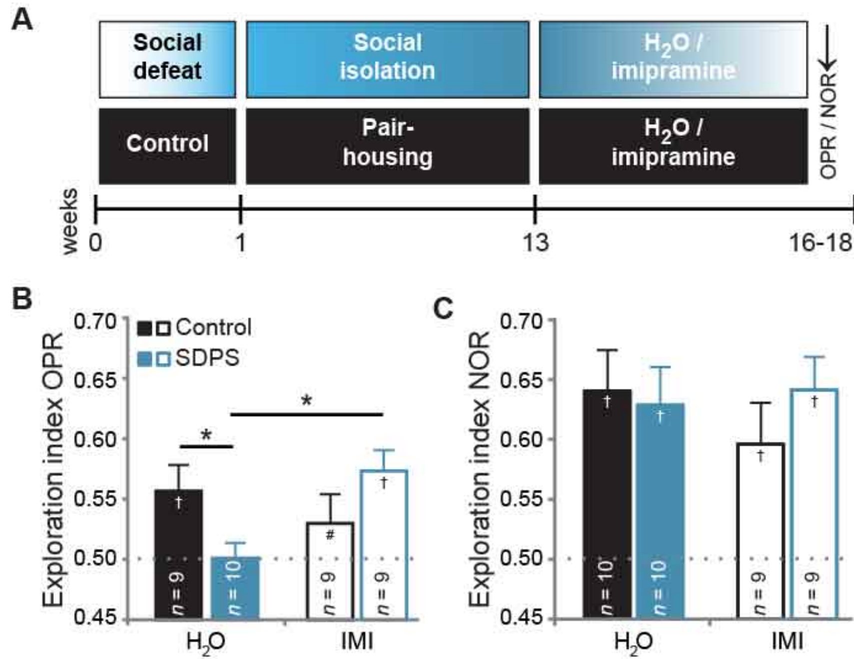
Author contributions

PvB, DR, WJGH, ABS, SS designed the proteomic experiments and biochemical validations; DR, PvB, RvdS executed the proteomic experiments and biochemical validations; DR, PvN, SS analyzed the proteomic experiments and biochemical validations; DR, ABS, SS designed the immunohistochemical experiments; DR, MKK, AdW executed and analyzed the immunohistochemical experiments; IK, PvB, DR, RMM, HDM, ABS, SS designed the physiological experiments; IK, AJT, TSH, PvB executed the physiological experiments; IK, SS, RMM, AJT, TSH, HDM analyzed the physiological experiments; PvB, JEvdH, DR, WJGH, ABS, SS designed the behavioral experiments; DR, PvB, JEvdH, executed behavioral experiments; DR,

PvB, JEvdH, SS analyzed behavioral experiments; DR, ANMS, ABS, SS designed the intervention (chondroitinase ABC) experiments; DR, YvM performed the intervention; DR performed the behavioral read-out of the intervention; MKK performed the immunohistochemical read-out of the intervention; IK, AJT, AWP executed the physiological read-out of the intervention experiment; DR, IK, MKK, AJT, SS analyzed the data for the intervention experiments; DR, ABS, SS wrote the manuscript.

Competing Interests: ABS and SS are coinventors on pending patent #P100640EP00 “Treatment of cognitive impairment in depressive disorders”. JEvdH is currently employed at Danone Nutricia Research Utrecht and at Noldus Information Technology Wageningen. ABS participates in a holding that owns shares of Sylics BV. PvB is currently employed as a business developer at the Neuroscience Campus AmsterdamVU Medical Center. The other authors declare no competing interests.

1 **Figures & legends**



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3 **Figure 1. SDPS induces deficits in rat spatial memory that are reversed by imipramine.**

4 **(A)** Rats were exposed to the social defeat-induced persistent stress (SDPS) paradigm, consisting

5 of 5 daily social defeat episodes and ~3 months of individual housing. Pharmacotherapy with

6 imipramine (IMI) or vehicle (H_2O) as control was applied during the last three weeks of the

7 isolation period in both groups. Rats underwent behavioral assessment using the object place

8 recognition (OPR) test (panel B) or the novel object recognition (NOR) test (panel C). **(B)**

9 Exploration index during the test phase of the OPR task. SDPS impaired memory retention of the

10 object location; imipramine reversed this effect but had no effect on control animals. **(C)**

11 Exploration index during the test phase of the NOR task. Neither SDPS nor imipramine treatment

12 affected recognition performance. Dotted line represents exploration at chance level (0.50);

13 n=number of animals; Two-way ANOVA; *post hoc* Fisher's LSD; **P*<0.05; (see Table S2).

14 †Significant memory retention (panel I) for *P*<0.05, and #trend for *P*<0.2 by unpaired t-test.

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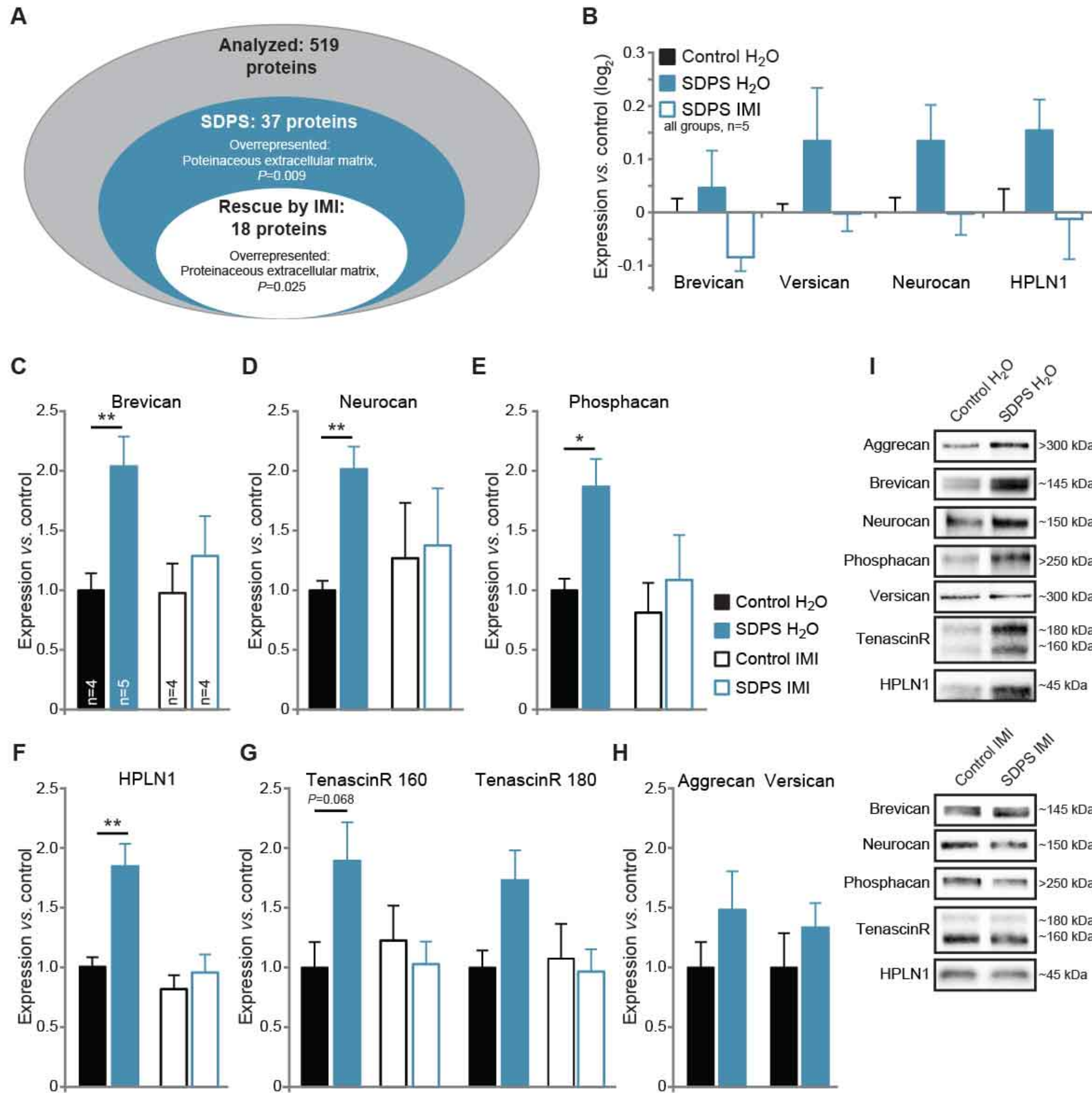

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2 **Figure 2. SDPS induces increased perisynaptic CSPG expression in the dorsal hippocampus**

3 **that is reversed by imipramine.**

4 **(A, B)** Proteomic analysis using iTRAQ of the dorsal hippocampal synaptic membrane fraction at

5 3 months after the last social defeat episode. The results revealed 37 SDPS-regulated proteins

6 (adjusted $P<0.05$; panel A). Expression of 18 of these proteins was rescued by treatment with

7 imipramine (IMI; adjusted $P<0.1$, SDPS-IMI *vs*. SDPS-H_2O; panel A). Extracellular matrix

8 (ECM) proteins, in particular chondroitin sulfate proteoglycans (CSPGs), were overrepresented in

both groups of 37 and 18 differentially expressed proteins (B). **(C-F)** Independent immunoblot analysis revealed that SDPS increased the synaptic expression of several CSPGs, including brevican (C), neurocan (D), phosphacan (E) and the PNN backbone protein hyaluronan and proteoglycan link protein 1 (Hapln1) (F). Imipramine (IMI) treatment reversed this effect. **(G,H)** Immunoblots for tenascinR (160 and 180 kDa; panel G), and aggrecan and versican (panel H) showed a moderate effect of SDPS on expression ($0.05<P<0.20$). **(I)** Example blots showing the effect of SDPS on protein expression and that imipramine treatment reversed this effect. The apparent molecular weight is indicated for the specific protein band; total protein loading used for normalization can be found in Fig. S4. n=number of samples; PLGEM (panels A, B); One-way ANOVA (panels C, D, F, G, H); Mann-Whitney (panel E); *$P<0.05$; **$P<0.01$ (see Table S2).

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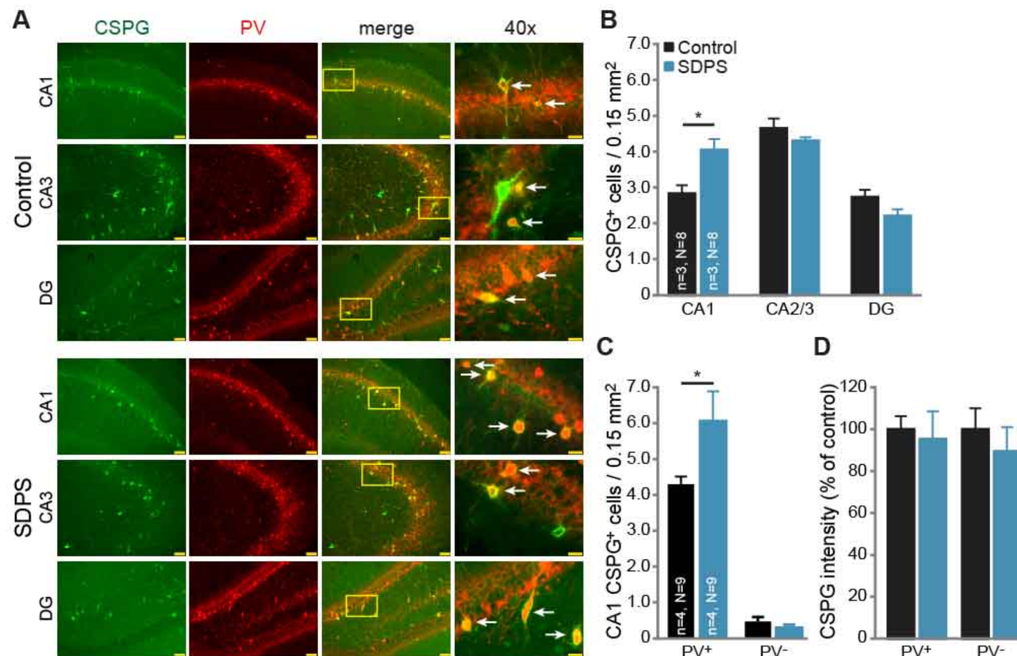

2 **Figure 3. SDPS increases the number of PNN-coated parvalbumin-expressing interneurons**

3 **in the hippocampus.**

4 **(A)** PNN-coated (PNN^+) parvalbumin-positive (PV^+) interneurons of the dorsal hippocampal

5 subfields were quantified for control *vs.* SDPS rats at 2 months after the last defeat. Double

6 immuno-positive interneurons (PNN^+ PV^+) in the hippocampus CA1 region are indicated by white

7 arrows in the 40x magnification images. (**B)** SDPS increased the number of PNN^+ cells in the CA1

8 region, but not in CA2/3 or the dentate gyrus (DG) regions of the hippocampus. (**C,D)** The increase

9 in PNN number was specific for PV^+ interneurons of the hippocampal CA1 stratum pyramidale

10 region (C), and was not accompanied by an alteration in PNN intensity (D). Scale bars in panel A:

11 75 µm, or 25 µm (40x); n=number of animals; N=number of sections; One-way ANOVA (panels

12 B, C); paired t-test (panel D); **P*<0.05 (see Table S2).

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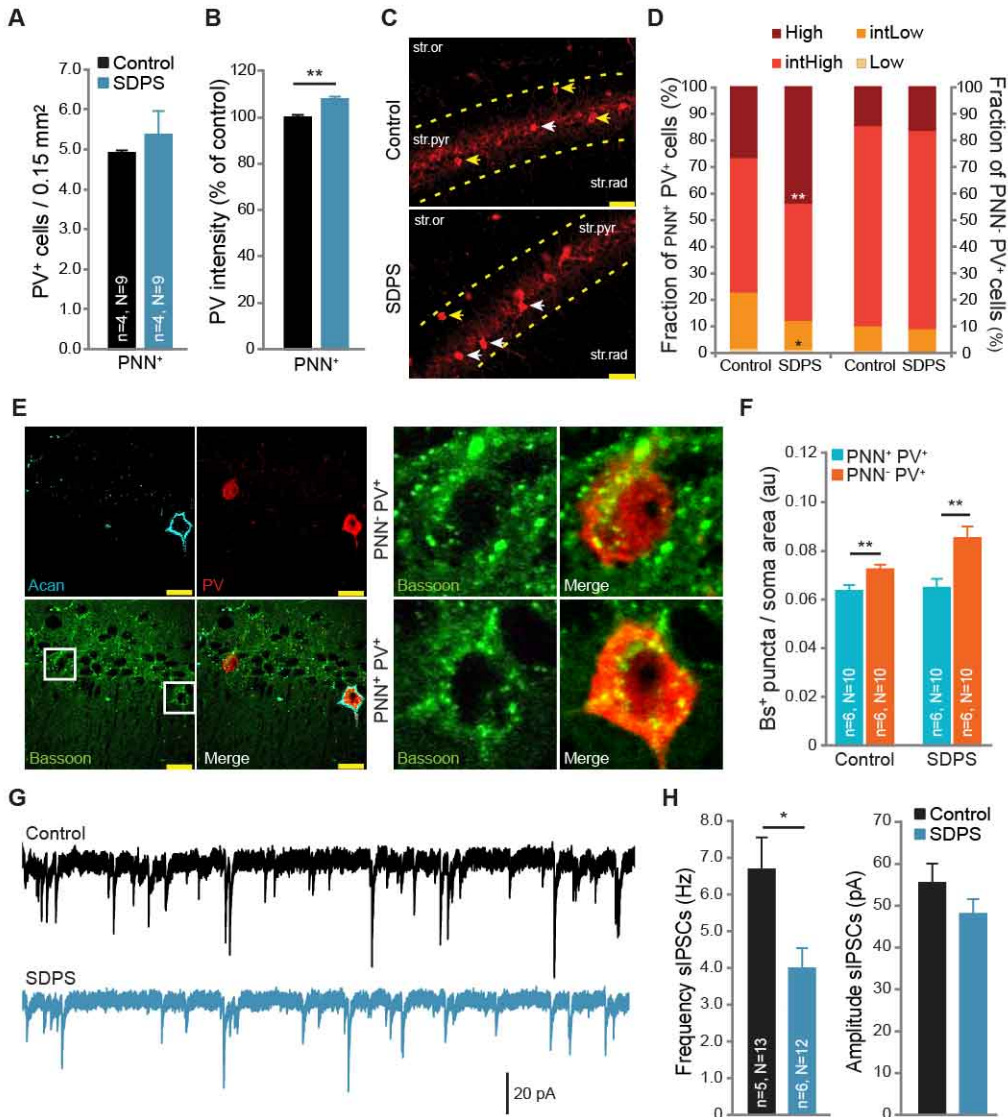

2 **Figure 4. SDPS alters parvalbumin-positive interneuron properties and decreases inhibitory**

3 **transmission in the hippocampus**.

4 **(A, B)** In the hippocampal CA1 stratum pyramidale, SDPS did not affect the total number of PNN-

5 coated parvalbumin-positive interneurons (PNN^+ PV^+) (panel A), but did cause a moderate

6 (7.8%±1.0) increase in the intensity of parvalbumin immunoreactivity in SDPS *vs.* control animals

7 (panel B). **(C, D)** Representative examples of labeling of hippocampal CA1 parvalbumin-positive

8 interneurons in SDPS and control animals (C; high intensity, white arrowheads; intermediate–low

9 intensity, yellow arrowheads). **(D)** Within double immuno-positive (PNN^+ PV^+) interneurons,

1 SDPS decreased the fraction of intermediate–low parvalbumin expressing cells (control, 22.1%;
2 SDPS, 11.8%) and increased the fraction of high parvalbumin expressing interneurons (control,
3 26.2%; SDPS, 44.1%; panel D, *left*). No difference in the fraction of low or intermediate-high
4 parvalbumin expressing interneurons was observed. No intensity-shift was observed in PNN-free
5 parvalbumin-positive (PNN^{-} PV^{+}) interneurons (panel D, *right*). (**E, F)** Quantification of bassoon-
6 positive (Bs^{+}) puncta showed no effect of SDPS on perisomatic excitatory input onto PNN^{+}/PV^{+}
7 interneurons. In control and SDPS animals alike, PNN^{-} PV^{+} interneurons showed increased density
8 of Bs^{+} puncta *vs*. PNN^{+} PV^{+} cells (F). (**G)** Example traces of whole cell patch-clamp recordings
9 (5 s) of hippocampal CA1 pyramidal neurons. (**H)** SDPS reduced sIPSC frequency (*left*), while
10 leaving amplitude unaffected (*right*). Scale bars indicate 50 μm (C) or 20 μm (E); str.or: stratum
11 oriens; str.pyr: stratum pyramidale; str.rad: stratum radiatum.; n=number of animals; N=number
12 of sections/slices; Mann-Whitney (panels A, F); Paired t-test (panels B, F); One-way ANOVA
13 (panels D, F); **P*<0.05, ***P*<0.01 (see Table S2).
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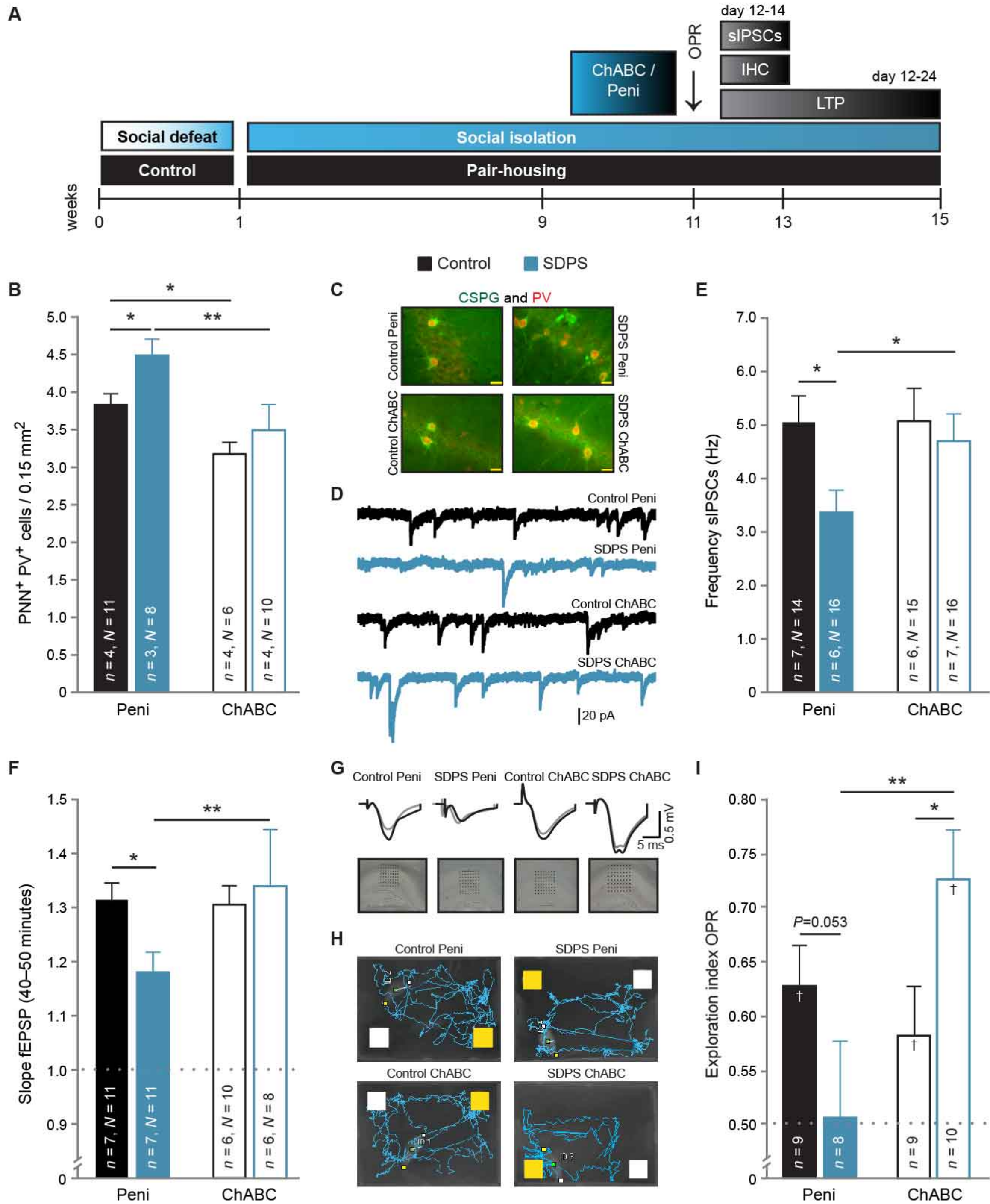

Figure 5. Intra-hippocampal chondroitinase ABC administration restores PNNs, hippocampus function and memory recall after SDPS.

(A) Following SDPS (or when control), animals received either intrahippocampal administration of chondroitinase ABC (ChABC) or Penicillinase (Peni) as a control. Performance on the object

place recognition (OPR) test was assessed 12 days post-administration (H,I) and was followed by LTP measurements at 12–24 days post-treatment (F,G). Immunohistochemistry (B,C)) and sIPSC recordings (D,E) were performed at 12–14 days post-treatment. (**B, C)** SDPS increased the number of double immuno-positive (PNN^+/PV^+) interneurons (representative example, panel C), and treatment with chondroitinase ABC reversed this effect. Chondroitinase ABC treatment reduced the number of PNN^+ PV^+ neurons compared to penicillinase treatment. (**D, E)** Frequency of sIPSCs (representative example traces, panel D) was reduced after SDPS, and chondroitinase ABC treatment rescued this effect. Chondroitinase ABC treatment had no effect on sIPSC frequency in control rats. (**F,G)** Maintenance of LTP, expressed as fEPSP slope, was decreased in SDPS rats and restored after chondroitinase ABC treatment. Chondroitinase ABC treatment had no effect on LTP maintenance in control animals. (**G)** Example of placement on the MED-64 grid with fEPSP traces before and after (grey/black, respectively) high frequency stimulation to induce LTP. **(H, I)** Rats exposed to the SDPS paradigm showed impaired object location memory on the OPR test, and chondroitinase ABC reversed this effect. **(H)** Example of animal movements during the OPR test. Yellow squares represent the displaced object. Scale bar in panel C indicates 25 μm. Dotted line represents baseline fEPSP slope before high frequency stimulation (F), or exploration at the chance level (0.50, I); n=number of animals; N=number of cells/sections; One-way ANOVA, *post-hoc* Fisher's LSD (panels B, E, F); two-way ANOVA, *post-hoc* Fisher's LSD (panel I); *P<0.05, **P<0.01 (see Table S2). †Significant memory retention (panel I) for P<0.05, and #trend for P<0.2 by unpaired t-test.

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