Offline ventral subiculum-ventral striatum serial communication is required for spatial memory consolidation

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The hippocampal formation is considered essential for spatial navigation. In particular, subicular projections have been suggested to carry spatial information from the hippocampus to the ventral striatum. However, possible cross-structural communication between these two brain regions in memory formation has thus far been unknown. By selectively silencing the subiculum-ventral striatum pathway we found that its activity after learning is crucial for spatial memory consolidation and learning-induced plasticity. These results provide new insight into the neural circuits underlying memory consolidation and establish a critical role for off-line cross-regional communication between hippocampus and ventral striatum to promote the storage of complex information.
Established models describing stabilization of episodic memory suggest that the hippocampus (HPC) plays a crucial role in the early stages of memory consolidation (see ref. 1). This view has recently evolved with the demonstration that memory consolidation depends upon the coordinated activity of the HPC and a network of brain regions including the neocortex and key subcortical structures2–4. In this framework, neural activity in the ventral striatum (VS) has recently emerged as a fundamental event supporting spatial learning and memory consolidation. Indeed VS post-training manipulations induce deficits in spatial memory tasks, suggesting the necessity of offline neural activity within this brain region in order to consolidate spatial memories5–8. In line with these findings, it has been demonstrated that key molecular alterations, required for successful consolidation of newly acquired spatial information in the HPC, also occurs in the VS9.

The VS receives prominent glutamatergic projections from the prefrontal cortex, the amygdala and the HPC formation10–13. It has been hypothesized that each one of these distinct pathways relays specific information to the VS. In particular, electrophysiological recordings in rodents suggest that HPC–VS projections might carry spatial information14,15. Disconnection experiments provide further support for a cross-regional interplay between HPC and VS in spatial and contextual information processing16–18. The fact that these manipulations were performed pre-training, however, leaves uncertainty on whether disruption of HPC–VS connections impairs encoding of spatial information or disrupts the consolidation of regularly encoded spatial information.

Relevant electrophysiological evidence showing off-line coherent reactivation of neuronal ensembles in the HPC and in the VS after learning19, supports the hypothesis that post-training cross-structural communication between these two brain structures might be a critical mechanism for long-term stabilization of spatial information. However, the causal role of the functional interplay between HPC and VS in memory consolidation remains to be proven. In this study by silencing subicular projections to the VS, we demonstrated that off-line serial communication between these two brain regions is needed for spatial memory consolidation and the development of learning induced structural changes necessary for long-term memory stabilization.

**Results**

**Learning-induced activation of ventral subiculum–VS projections.** To provide direct evidence supporting the hypothesis that HPC–VS communication is required for spatial memory consolidation, we started exploring spatial learning-induced neuronal activation in HPC projections to VS, combining retrograde tracing with cell-activity dependent labeling in mice trained in the spatial version of the Morris water maze (sMWM). One week after Fluoro-Gold (FG) administrations in VS, mice were trained in the sMWM using a single-day massed procedure. Control groups were either naïve to training or trained in the cue version of the maze (cMWM) (Fig. 1a). Retrograde FG tracing confirmed the ipsilateral ventral subiculum (vSUB) as the major source of HPC projections to VS (Fig. 1b; Supplementary Fig. 1b, c)10. A few labeled neurons were found also in the dorsal subiculum and the ventral CA1 (Fig. 1b). Importantly, we found no contralateral subicular projections (Supplementary Fig. 1b). Interestingly, fos expression was significantly increased in the entire vSUB, as well as in the subicular VS-projecting cells, after both cue and spatial learning compared to naïve mice (Fig. 1c, d) indicating that vSUB is activated regardless of the navigational strategy20.

**vSUB–VS activation is not necessary for cue-memory.** Cell activity-dependent labeling in the vSUB–VS projection after cue learning suggests a role of the pathway independent of the type of information being consolidated. To verify this possibility, an additional control group was injected in the vSUB and in the contralateral VS immediately after training in the cMWM. Mice administered AP-5 were able to locate the platform 24 h after training, compared to vehicle controls (Fig. 2a; Supplementary Fig. 3a, c). On the contrary, a control group receiving the NMDA antagonist ipsilaterally in the two brain regions showed an intact ability to reach the platform compared to vehicle-injected mice (Fig. 2b; Supplementary Fig. 3d, f). To test the generality of this finding, we assessed the effects of the off-line vSUB–VS functional disconnection on spatial memory performance in an object displacement task (ODT) (Fig. 2c). As previously observed in the sMWM, the AP-5 disconnected group showed an impaired ability to discriminate the spatial change compared to vehicle-injected controls (Fig. 2c; Supplementary Fig. 5).

**vSUB is required for spatial learning-induced VS plasticity.** We have recently demonstrated that increased spine density in the VS accompanies the consolidation of spatial memories22. Having shown off-line interference with communication between the vSUB and the VS impairs spatial memory, we wondered whether there was a role for this connection in learning-induced structural plasticity in the VS. Mice were trained in the sMWM and immediately post-training injected unilaterally with AP-5 in vSUB. The contralateral vSUB was injected with vehicle as an internal control (Fig. 3a; Supplementary Fig. 8). To verify the specificity of the effect, further groups of animals were trained in the cMWM (Supplementary Fig. 8). A group of naïve mice was used to normalize data obtained from cue and spatial trained
mice. Spine counts in VS 24 h after training demonstrated a spatial learning-induced specific increase in spine density, that was impaired by blockade of vSUB activity (Fig. 3b). This result confirms our early findings of increased spine density in VS after spatial learning22, and validates the hypothesis that vSUB neuronal activity is required to promote learning-induced structural changes in the VS.

**Inhibition of vSUB–VS projections impairs spatial memory.** To determine whether the effect of vSUB–VS pathway disconnection observed was due to direct projections from the vSUB to the VS, we used the hM4D1–clozapine-N-oxide (CNO) chemogenetic neural inhibition tool to rapidly and selectively inhibit vSUB–VS projecting neurons (Fig. 4a, e). We first examined whether activation of hM4D1 in VS-projecting subicular terminals by local CNO delivery could modulate the activity of VS neurons by recording spontaneous excitatory postsynaptic currents (sEPSCs) from medium spiny neurons (MSNs) in VS slices of a cohort of mice (n = 6) injected with the viral vector in the vSUB (Fig. 4a). In 10 out of 13 cells recorded, bath-application with 10 μM CNO decreased the frequency (Fig. 4b, c) but not the amplitude of sEPSCs (Fig. 4b, d), indicating that hM4D1, DREADDs activation in VS-projecting subicular terminals decreased glutamate release onto MSNs in the VS. The latency of the CNO-mediated effect on the EPSCs frequency was 9.30 ± 1.1 min. The analysis of the resting membrane potential (Vm), the input resistance (Rinput) and the firing frequency (in response to depolarizing current steps; 800 ms duration, 300 pA amplitude) did not show differences before and during CNO application (Supplementary Fig. 9a–d). CNO application did not affect either the frequency or the amplitude of EPSCs (Supplementary Fig. 9e, g) recorded in spiny neurons (n = 8) obtained from naïve mice (n = 5).

Next a cohort of mice was bilaterally implanted with guide cannulae above the VS after viral vector injection in the vSUB (Fig. 4e), to allow the selective manipulation of the VS-targeting subicular fibers (Fig. 4f; Supplementary Fig. 10). All mice underwent training in the MWM (Supplementary Fig. 11a, b) and were administered with vehicle or CNO 10 mM23 immediately after training. The probe test performed 24 h later revealed that CNO-injected mice spent significantly less time in the target quadrant compared to vehicle-injected mice (Fig. 4g). To establish the temporal dynamic of the involvement of vSUB–VS projection in spatial memory consolidation, we performed an additional experiment administering CNO in the VS 120 min after training. Both vehicle and CNO administered mice were able to correctly locate the platform (Fig. 4h; Supplementary Fig. 11d–f), thus demonstrating that the activity of this pathway has a time-limited role for spatial memory consolidation. VS CNO administrations in independent groups of mice demonstrated that activation of hM4D1 in VS-projecting subicular terminals was void of significant effects on anxiety, locomotion and novelty (Supplementary Fig. 12). Further control experiments ruled out also possible effects of CNO alone on learning, locomotor activity or anxiety (Supplementary Fig. 13). These data confirmed the need for post-training cross-regional communication between the vSUB and the VS to consolidate spatial information, and demonstrated also that this effect is mediated by direct projections between the two structures. Overall, our results reveal that spatial memory consolidation and related structural plasticity depend upon off-line communication between the vSUB and the VS in the early stages after learning.

**Discussion**

Focusing on the early stages of memory consolidation, our findings establish that neural communication between HPC and
Fig. 2 vSUB-VS pathway disconnection impairs spatial memory consolidation. a Immediate post-training functional disconnection between the vSUB and the VS with AP-5 (n = 10), impaired spatial memory consolidation compared to vehicle controls (n = 9) (two-way ANOVA of quadrant preference F3.51 = 15.824; p = 0.0001; treatment F1.17 = 0.270; p = 0.6100; quadrant × treatment F3.51 = 4.104; p = 0.0110). The insert shows first bearing of mice in the two groups (t17 = 3.450; p = 0.0031; unpaired t-test). Bottom panels: representative path from vehicle and AP-5 animals. b Unilateral post-training AP-5 (n = 10) administrations in the vSUB and in the ipsilateral VS did not impair mice ability to locate the platform compared to vehicle-injected mice (n = 11) (two-way ANOVA of quadrant preference F3.57 = 20.775, p = 0.0001; treatment F1.19 = 1.077, p = 0.3124; quadrant preference × treatment F3.57 = 0.499, p = 0.6846). The insert shows first bearing of mice in the two groups (t19 = 0.709; p = 0.4871; unpaired t-test). Bottom panels: representative path from vehicle and AP-5 animals. c Immediate post-training vSUB-VS functional disconnection (n = 9) impaired mice ability to discriminate spatial displacement 24 h after training, compared to vehicle-injected mice (n = 15) (vehicle: session × objects F1.8 = 5.34, p = 0.036; AP-5: session × objects F1.14 = 0.37; p = 0.55). Histograms represent mean ± SEM; *p < 0.05 displaced objects (DO) in S5 vs DO in S4 (within group, Fisher). d Post-training functional disconnection between vSUB and VS (n = 9) did not affect mice ability to locate the platform 24 h after training in the cMWM (vehicle n = 8) (two-way ANOVA of quadrant preference F3.45 = 14.814; p = 0.0001; treatment F1.15 = 1.134; p = 0.3037; quadrant preference × treatment F3.45 = 0.369; p = 0.7754). The insert shows first bearing of mice in the two groups (t15 = 0.299; p = 0.7692; unpaired t-test). Bottom panels: representative path from vehicle and AP-5 animals. Histograms represent mean ± SEM; *p < 0.05 target vs right, opposite, left (within group); #p < 0.05 target vs target (between groups, Tukey). MWM images modified from ref. 9; Image credit for brains schematics: Allen Institute.

VS is required immediately after learning to properly store spatial information and to promote in the VS the structural changes underlying it.

In particular, the experiments presented provide direct indication that activity in the two brain regions is not occurring independently or simultaneously, but rather neural activity in the vSUB is driving neuronal activation in the VS and this cross-structural interaction is a key step for spatial memory consolidation. It is worth noting that in all the experiments presented, we performed post-training manipulations, thus
suggesting that active communication between the two brain regions is needed after training. Our data are consistent with prior correlative evidence showing that memory in the early stages of consolidation is sustained by replay in connected brain areas\(^{19}\) and provide support to a causal role of this communication to long-term storage of spatial information.

The VS receives glutamatergic projections from the HPC, the amygdala and the prefrontal cortex\(^ {15,24-26} \). This circuit involves also trans cortical networks connecting the HPC to the VS indirectly through the amygdala or the prefrontal cortex\(^ {27-29} \). Interestingly, activation of different projections leads to distinct physiological and behavioral responses\(^ {16,18,30,31} \). Different aspects of spatial navigation seem to depend on the activity of separate limbic-cortical-striatal circuits\(^ {18} \). Direct projection from the HPC to the VS would guide navigation in novel environment independent of previous knowledge. On the contrary, indirect projection through the prefrontal cortex would guide prospective navigation dependent on previously acquired information\(^ {18} \). Our chemogenetic data challenge this view by demonstrating that consolidation of spatial memory, necessary to perform a delayed recall, depends on direct projection from the vSUB to the VS.

Current models support the occurrence of changes in neuronal morphology and connectivity after spatial learning in the HPC and at later interval in the neocortex\(^ {32,33} \). Our findings demonstrate that such changes, early after encoding, occur also in the VS, are specifically induced by spatial training and that immediate post-training activity of the subicular input is a necessary requirement. These observations, further support a specific role of the VS and the subicular projection to this structure in explicit memory. They also demonstrate the occurrence of such changes in brain regions outside the medial temporal lobe immediately after the learning experience. Although to our knowledge, demonstration of spatial learning-induced structural plasticity outside the medial temporal lobe immediately after the learning experience\(^ {18} \) (two-way ANOVA of training \( F_{1,18} = 18.162, p = 0.0005; \) treatment \( F_{1,18} = 8.056, p = 0.0109; \) training × treatment \( F_{1,18} = 5.329, p = 0.0331)\). Scale bar: 5 μm. Histograms represent mean ± SEM; * \( p < 0.05 \) spatial vs cue (Tukey).

By showing that immediately after learning, off-line serial neural communication between the HPC and the VS is required for the storage of a spatial, but not a cue-based memory, our data identify in the offline HPC-VS interplay a dynamic serial interaction between the two regions specific to spatial memory consolidation. The VS is generally viewed as the downstream structure performing action selection based on HPC and prefrontal cortical input\(^ {35-38} \). Recent network analysis, on the contrary, locate the VS upstream the HPC and the prefrontal cortex, playing a central role in updating memories based on new information\(^ {3} \). Although the present study did not specifically investigate this issue, demonstrating control of vSUB projections over VS in spatial memory consolidation, it seems to support the first hypothesis. Moreover, it may represent a mechanism by which VS gates inappropriate responses based on previous experience\(^ {38} \). Nevertheless, regardless the models considered, our results demonstrate the necessity for VS activity in HPC-dependent memories and a possible mechanism by which different parts of the brain integrate their activity to store relevant information.

**Methods**

**Subjects.** All experiments were conducted in naïve CD1 male mice (Charles River, Italy), 10-15 weeks old and weighing 35-50 g at the start of the experiments. Animals were housed in groups of four in standard cages (26.8 × 21.5 × 14.1 cm) with enrichment conditions, water and food ad libitum, under 12 h light/dark cycle and constant temperature (22 ± 1 °C). Behavioral training and testing were conducted during the light period (from 9:00 am to 5:00 pm). All the animals were treated in respect to current Italian and European laws for animal care, and the maximum effort was made to minimize animal suffering.

**Stereotaxic surgery.** Each mouse was deeply anesthetized with chloral hydrate (500 mg/kg; Sigma Aldrich, Italy) and secured on a stereotaxic apparatus (David Kopf Instruments, USA). Two stainless-steel guide cannulae (0.50/0.25 × 7 mm; Unimed, Switzerland) were implanted as follows, depending on the experiment: (1) bilaterally in vSUB; (2) bilaterally in the VS; (3) contralaterally in one vSUB and one VS; (4) ipsilaterally in one vSUB and one VS. Cannulae implantation was performed through craniotomies on the skull at the following coordinates relative to bregma: antero-posterior (AP) = −3.4 mm, medio-lateral (ML) = ± 3 mm, dorso-ventral (DV) = −1.3 mm from bregma for the vSUB; AP = ± 1.7 mm, ML = ± 1 mm, DV = −1.3 mm from bregma for the VS. Guide cannulae were anchored to the skull through the use of dental cement (Illic, Italy). Mice were allowed to recover for at least 1 week after surgery.
Fig. 4 Inhibition of vSUB terminals targeting the VS impairs spatial memory consolidation. a Schematic of the ex vivo electrophysiology DREADDs experiment. b Representative traces from VS MSNs showing sEPSCs in control conditions (black) and under CNO (orange). c Scatter plot graph summarizing sEPSC frequency in control conditions (black) and under CNO (orange) (n = 13 cells/6 mice; t_{12} = 2.8, p = 0.015; paired t-test). d CNO did not change the sEPSCs amplitude (p = 0.38; paired t-test). e Schematic of the behavioral DREADDs experiment. f Schematic of AAV expressing Venus and the HA-tagged hM4Di under the h-Synapsyn (Syn) promoter. Representative coronal sections from HPC and VS. Scale bars: 1 mm. g Mice infected in the vSUB with the AAV and injected post-training with CNO (10 mM) in the VS showed impaired performance on the probe test. Histograms represent the mean of distance traveled in each quadrant for vehicle (n = 7) and CNO (n = 13) injected groups (repeated measures ANOVA of quadrant preference F_{3,54} = 22.976, p = 0.0001; treatment F_{1,18} = 0.085, p = 0.7735; quadrant preference × treatment F_{3,54} = 8.019, p = 0.0002). The insert shows first bearing of mice in the two groups (t_{17} = 3.450; p = 0.0033; unpaired t-test). Bottom panels: representative path from vehicle and CNO animals. h Mice bilaterally infected in the vSUB and injected 120 min post-training with CNO (10 mM) in the VS showed no impairment in the performance. Histograms represent the mean of distance traveled in each quadrant for vehicle (n = 11) and CNO (n = 9) injected groups (repeated measures ANOVA of quadrant preference F_{3,54} = 24.6954, p < 0.0001; treatment F_{1,18} = 0.4137; p = 0.6178; unpaired t-test). Bottom panels: representative path from vehicle and CNO animals. Histograms represent mean ± SEM. *p < 0.05 target vs target (between groups); **p < 0.05 target quadrant vs right, opposite, left (within group, Tukey). Bottom panels: representative probe-trial. MWM images modified from ref. 9; Image credit for brains schematics: Allen Institute.
For retrograde tracing experiment, 0.25 μL of FG (4% in NaCl 0.9%) were injected unilaterally in the VS at −4.3 mm from the dura. The injector was left in place for an additional 5 minutes to allow diffusion. Animals were left to recover for about 7–9 days to allow FG retrograde diffusion.

Adeno-associated viruses (AAV1/2) were used to express the Venus fluorescent protein and human influenza hemagglutinin (HA)-tagged hM4D, (AAV-Syn:Venus-2A-HA-hM4D) in the vSUB, a designer Gq-coupled receptor activated exclusively by CNO23. Viral vector was a generous gift of Cornelius T. Gross. The AAV was inoculated bilaterally in the vSUB at −4.3 mm from the dura. The volume of injection was 0.3 μL per side, delivered with the use of glass pipettes. Pipettes were left in place for 3 additional minutes after injection to allow diffusion39. Animals were allowed to recover for about 4 weeks (to allow the expression and anterograde diffusion of the virus). After the recovery period, mice underwent bilateral cannulation in the vSUB as described above.

**Drugs and in vivo focal injection procedure.** General infusion procedure was performed for all experiments immediately after the end of the last session of training. A total of 0.25 μL of 0.6 μg/μl40,41 NMDA receptor antagonist (+)-2-amino-5-phosphonopentanoic acid (AP-5; Sigma-Aldrich, Italy), was bilaterally infused into the vSUB at −4.3 mm from the dura. The injection was left in place for 30 additional seconds to allow diffusion. A total of 0.30 μL of CNO (Cayman Chemical, USA) at 10 μM was dissolved in dimethyl sulfoxide (DMSO) and PBS solution (1:1). The dose was chosen based on previous literature. CNO was dissolved in phosphate-buffered saline (PBS 0.1 M). Control mice were identically injected with the same volume of PBS. After administration, the injector was left in place for 30 additional seconds to allow diffusion. The dose of CNO was kept constant in all experiments. Mice were deeply anesthetized with an overdose dose of anesthetic and transcardially perfused with 4% paraformaldehyde solution in PBS (4 °C). Free-floating sections 40 μm thick underwent 1h blocking step (5% Normal Goat Serum and 0.1% Triton X-100 in PBS) and overnight incubation with primary antibody against CNO (1:300; sc-52, Santa-Cruz Biotechnology, USA) at 4 °C, followed by washes and 1h incubation with fluorochrome-labeled secondary antibody (1:400, Rhodamine Red-X). Immunoreactivity was visualized using a microscope (Nikon, Eclipse FN1) equipped with a CCD camera (Scientifica, UK). Whole-cell voltage-clamp recordings were performed using a MultiClamp 700B amplifier (2 kHz low-pass filter and 10 kHz digitization) with pClamp 10.3 software (Molecular Devices, USA). Venus+ terminals expressing hM4D, were visualized by a 437 nm LED (pE1, Cool LED, UK). MSNs in the medial VS were identified using the AAV expression and spines. To this aim, 24 h after training, naïve, cue and spatial trained mice were deeply anesthetized with an overdose dose of anesthetic and transcardially perfused with 50 μL of 0.9% NaCl at room temperature. Brains were collected and impregnated using a Polyclone solution (1% potassium dichromate, 1% mercuric chloride and 0.8% potassium chromate) for 6 days41. vSUB slices were cut for placement verification, which was made on mounted slices without additional staining.

**Behavioral procedures.** The spatial water maze task (sWMW) consisted of three different phases distributed across three consecutive days: Familiarization, Training and Test35. Familiarization consisted of one session of three consecutive trials (intertrial interval: 20 s). No cues were attached to the wall and a 9-cm-diameter black bar was attached to the wall of the open field, always in the same position. Four identical objects were used in this task. The procedure consisted in five sessions. Briefly, in session 1 (S1, familiarization), the animals were individually placed in the center of the empty open field for a 6-min session to familiarize with the apparatus. Subjects were then removed and placed back in a holding cage. After 2 min inter-trial interval, they were placed in the open field (with objects) for three consecutive 6-min sessions (training phase sessions 2–4; S2–S4) separated by a 2-min interval, during which the animals were returned to the holding cage. During the training phase, the configuration of the objects was the same and the mice were introduced in the open field facing the wall, always from a different position. Twenty-four hours after training, another spatial challenge (S5) in which two of the objects (displaced objects—DO) were repositioned as compared to previous sessions. Mice were placed in the open field facing the wall, from a novel position never used during training.

**fos and FG co-localization.** For FG-immunofluorescence procedure mice were deeply anesthetized with an overdose dose of anesthetic and transcardially perfused with 40 mL of 0.9% NaCl solution (room temperature), followed by 40 mL of 4% paraformaldehyde solution in PBS (4 °C). Fresh-floating sections 40 μm thick underwent 1h blocking step (5% Normal Goat Serum and 0.1% Triton X-100 in PBS) and overnight incubation with primary antibody against fos (1:300; sc-52, Santa-Cruz Biotechnology, USA) at 4 °C, followed by washes and 1h incubation with fluorochrome-labeled secondary antibody (1:400, Rhodamine Red-X). A subset of 4 slices was acquired with a fluorescent microscope with a 4x magnification. A mosaic of the ipsi- and contralateral FG-immunoreacted hemispheres and the corresponding vSUB were manually acquired and stitched with FIJI (ImageJ, N.I.H., USA) and analyzed for FG diffusion. Two representative images of the VS and the vSUB were treated for nuclear counterstaining with VECTASHIELD® Antifade Mounting Medium with propidium iodide (PI) (Vector Laboratories, USA). PI-stained slices were acquired at the Olympus X83-FV1200 confocal laser scanning microscope with a 10χ NA 0.40 objective, 559 nm laser/PI setting for PI detection. Single images of 1600 x 1600 pixels were stitched together in a mosaic view with the Fiji-3D Viewer tool (Olympus Fluvoir 4.2). fos and PI images were acquired with a confocal microscope (Leica TCS-SP5) with a 20χ/0.64 objective in the vSUB. GF fluorescence was detected under 405 nm Diode laser, while fos under 453 nm HeNe laser. Approximately four to six consecutive 1024 pixels image sets were recorded over 4 weeks (to allow the expression and anterograde diffusion of the virus). After the recovery period, mice underwent bilateral cannulation in the vSUB as described above.

**Golgi-Cox staining.** Golgi-Cox staining was used to highlight dendritic arborization and spines. To this aim, 24 h after training, naïve, cue and spatial trained mice were deeply anesthetized with an overdose dose of anesthetic and transcardially perfused with 50 mL of 0.9% NaCl at room temperature. Brains were collected and impregnated using a Golgi-Cox solution (1% potassium dichromate, 1% mercuric chloride and 0.8% potassium chromate) for 6 days41. Slices were cut for placement verification, which was made on mounted slices without additional staining.

**Acute slice recordings.** Five to eight weeks after AAV injections in the vSUB were deeply anesthetized with an intraperitoneal injection of an overdose of anesthetic (a mixture of tiletamide/zolazepam, zoletil 80 mg/kg, and xilazine 10 mg/kg) and transcardially perfused with ice-cold artificial cerebrospinal fluid (ACSF). Brains were sectioned at the level of the VS in 250-μm-thick coronal sections on a VT-1200 vibratome (Leica, Germany). The ACSF used to both perfuse the animals and slice the brains was modified to contain (in mM): 75 sucrose, 87 NaCl, 2.5 KCl, 1.25 NaH2PO4, 7 MgCl2, 26 NaHCO3, 11 glucose and 3 sodium ascorbate. Slices were placed in a holding chamber filled with room temperature ACSF saturated with 95% O2 and 5% CO2 containing (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 1 MgCl2, 2.4 CaCl2, 26 NaHCO3, 11 glucose and 1 sodium ascorbate (310–315 mOsm). After incubation for at least 45 min, an individual slice was transferred to a submerged recording chamber and places in the recording chamber (World Precision Instruments, Inc.). The ACSF was continuously bubbled with 95% O2 and 5% CO2. Recording setup was designed to monitor spiking activity. To this aim, the head of the spine to the shaft of the dendrite were counted as spines. The number of spines per neuron from each individual were averaged and counted as single sample, and subsequently the final values per animal were averaged per group (naïve, cue and spatial trained). Values for cue and spatial trained were normalized on naive values separately for each replicate of the experiment. All measurements were performed by an experimenter blind to the experimental conditions.
current clamp configuration were performed on the same spiny neurons in order to evaluate their resting membrane potentials (Vrest), input resistance (Rin), and firing mode and control currents and during perfusion with CNO (10 μM). Repetitive hyperpolarizing and depolarizing current pulses (800 ms duration, 50 pA incremental amplitude) were delivered every 2 s through patch pipette at resting membrane potential. Measurements of membrane potentials were not corrected for the liquid junction potential error. Drugs were applied in the bath by gravity changing the perfusion solution to one differing only in its content of drug(s). The ratio of flow rate to bath volume ensured complete exchange within 2–3 min.

EPSCs were analyzed using pClamp software, which uses a detection algorithm based on a sliding template. The template did not induce any bias in the sampling of events because it was moved along the data trace by one point at a time and was optimally scaled to fit the data at each position. The detection criterion was calculated from the template-scaling factor and from how closely the scaled template fitted the data. Detection parameters were set at an amplitude >4 pA, and acquired events were visually inspected before averaging. A minimum of 100 sEPSCs was analyzed during 3 min of the baseline period and 3 min during CNO perfusion. Rinp, values were obtained from ΔΔ measured in response to hyperpolarizing current steps (800 ms duration, −50 pA).

Additional methods are given in the Supplementary information.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**References**

1. Bird, C. M. & Burgess, N. The hippocampus and memory: insights from spatial processing. *Nat. Rev. Neurosci.* 9, 182–194 (2008).
2. Vetere, G. et al. Chemogenetic interrogation of a brain-wide fear memory network in mice. *Neuron* 94, 363–374.e4 (2017).
3. Del Ferraro, G. et al. Finding influential nodes for integration in brain networks using optimal percolation theory. *Nat. Commun.* 9, 1–12 (2018).
4. Park, H. J. & Friston, K. Structural and functional brain networks: from connections to cognition. *Science* 342, 1238411 (2013).
5. Setlow, B. & McGaugh, J. L. Differential effects of immediate posttraining subpride microinfusions into the nucleus accumbens shell and core on Morris water maze retention. *Psychobiology* 27, 248–255 (1999).
6. Roulet, P., Sargolini, F., Oliverio, A. & Mele, A. NMDA and AMPA antagonist infusions into the ventral striatum impaire different steps of spatial information processing in a nonassociative task in mice. *J. Neurosci.* 21, 2143–2149 (2001).
7. Sargolini, F., Florian, C., Oliverio, A., Mele, A., & Roulet, P. Differential involvement of NMDA and AMPA receptors within the nucleus accumbens in consolidation of information necessary for place navigation and guidance strategy of mice. *Learn. Mem.* 10, 285–292 (2003).
8. Ploeger, G. E., Spruit, B. M. & Cool, A. Spatial localization in the Morris water maze in rats: Acquisition is affected by intra-accumbens injections of the dopaminergic antagonist haloperidol. *Behav. Neurosci.* 108, 927–934 (1994).
9. Ferretti, V. et al. Ventral striatal plasticity and spatial memory. *Proc. Natl Acad. Sci. USA* 107, 7945–7950 (2010).
10. Groenewegen, H. J., Vermeulen-Van der Zee, E., te Korthorst, A. & Witter, M. P. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of phasodus vulgaris leucoagglutinin. *Neuroscience* 23, 103–120 (1987).
11. Kelley, A. & Domescik, V. The distribution of the projection from the hippocampal-formation to the nucleus accumbens in the rat: an anterograde- and retrograde-horseradish peroxidase study. *Neuroscience* 7, 2321–2335 (1982).
12. Brog, J. S., Salyapongse, A., Deutch, A. Y. & Zahn, D. S. The patterns of afferent innervation of the core and shell in the ‘accumbens’ part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J. Comp. Neurol.* 338, 255–278 (1993).
13. Brit, J. P. et al. Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. *Neuron* 76, 790–803 (2012).
14. Olson, M. N., Kretz, J. A., Shalit, S. & Bolam, J. P. Nuclear accumbens: a functional and anatomical interface between the sensorimotor and the cerebral cortex. *J. Neurosci.* 28, 2843–2854 (2011).
41. Florian, C. & Roullet, P. Hippocampal CA3-region is crucial for acquisition and memory consolidation in Morris water maze task in mice. Behav. Brain Res. 154, 365–374 (2004).
42. De Leonibus, E., Oliverio, A. & Mele, A. A study on the role of the dorsal striatum and the nucleus accumbens in allocentric and egocentric spatial memory consolidation. Learn. Mem. 12, 491–503 (2005).
43. Pignataro, A., Borreca, A., Ammassari-teule, M. & Muldei, S. CREB regulates experience-dependent spine formation and enlargement in mouse barrel cortex. Neural Plast. 2015, 651469 (2015).

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Author contributions
G.T. carried out, designed and analyzed all the experiments, except for the electrophysiology experiments, which were carried out and analyzed by M.G.; chemogenetics experiments were tested by L.A., V.K., V.M. and E.C.; A.P. collaborated for the spine analyses; G.M.B. and L.A. for the pharmacology experiments, V.d.T. for the virus expression analysis. The project was conceived by G.T. and A.M. with critical input from A.R. and M.A.-T. The manuscript was written by G.T. and A.M. with inputs from A.R. and M.A.-T.

Competing interests
The authors declare no competing interests.

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