Supplemental Material

Reagents

Rabbit anti-phospho-S6 (Ser235/236, #2211S; 1:1000 for western blot; 1:500 for IHC), anti-S6 (#2217S, 1:3000) and anti-CRMP2 (#9393S; 1:2000) antibodies were purchased from Cell Signaling Technology (Danvers, MA). Mouse anti-NeuN (#MAB377, 1:500), rabbit anti-GluA1 (#MAB04-855, 1:500) antibodies, and nitrocellulose membranes were purchased from EMD Millipore (Billerica, MA). Rabbit anti-GAPDH (#sc-25778, 1:2000), mouse anti-PSD-95 (#sc-32290, 1:2000), anti-Arc (#sc17839, 1:1000) and goat anti-Homer (#sc-8921, 1:2000) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-Prosapip1 antibodies (24936-1-AP; 1:1000) were obtained from Proteintech (Rosemont, IL). Donkey anti-rabbit, anti-mouse and anti-goat horseradish peroxidase (HRP) antibodies were purchased from Jackson ImmunoResearch (West Grove, PA). NuPAGE Bis-Tris precast gels, donkey anti-rabbit IgG AlexaFluor 594 (#A21207), and donkey anti-mouse AlexaFluor 488 (#A21202) were purchased from Life Technologies (Grand Island, NY). EDTA-free complete mini Protease Inhibitor Cocktails were purchased from Roche (Indianapolis, IN). Phosphatase Inhibitor Cocktails 2 and 3 were from Sigma Aldrich (St. Louis, MO). Enhance Chemiluminescence (ECL) was from GE Healthcare (Buckinghamshire, UK). Pierce Bicinchoninic Acid (BCA) protein assay kit was obtained from Thermo Scientific (Rockford, IL) and BioMax MR Films were purchased from Kodak (Rochester, NY). Ethyl alcohol (190 proof) was purchased from VWR (Radnor, PA), Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich and rapamycin (#R-5000) was purchased from LC Laboratories (Woburn, MA). (R)-lacosamide was synthetized as described previously by (Choi et al., 1996).

Preparation of solutions

Alcohol (0.9 and 1.8 g/kg) was diluted in 0.9% saline to 20% (v/v). Rapamycin (10 mg/kg) was dissolved in 100% DMSO and administered in a volume of 2 ml/kg (Neasta et al., 2010). Lacosamide (10 mg/kg) was dissolved in 0.1% DMSO in saline and administered in a volume of 10 ml/kg. The vehicles used for control treatments are 100% DMSO and 0.1% DMSO in saline solution for rapamycin and lacosamide treatments, respectively. All treatments were administered intraperitoneally (i.p).

Conditioned place preference procedures

Acquisition of alcohol -induced conditioned place preference

The protocol used to acquire alcohol CPP was performed accordingly to (Neasta et al., 2010). The CPP apparatus (Columbus Instrument) consisting of a rectangular Plexiglas box (length 42 cm, width 21 cm and height 21 cm) divided by a central partition into two equal chambers (21 × 21 × 21 cm) equipped with horizontal photo beams was housed in a dark sound-attenuating box. The compartments of the CPP apparatus are distinguished by the wall color (black vs. white) and the rough textured floor (ribbed vs. dotted). During the conditioning trials the individual compartments were closed off from each other and during the test sessions, the central partition was elevated to 4 cm above the floor of the apparatus, allowing the mice to enter both sides of the apparatus. The time spent in each of the two compartments of was quantified by an automated system (Optomax, Columbus Instrument) on test days. All tests were conducted during the light phase of the 12h light/dark cycle in a quiet room dimly illuminated (30 lux). Prior to the beginning of the alcohol place preference conditioning phase, each animal was handled for 1 min per day and habituated to i.p. injection of a saline solution once a day for three consecutive days. During day 1, the initial preference was assessed (Fig. 1A, *pre-acquisition*

test). The next day, conditioning training started with one conditioning trial per day for 6 days. Mice were administered (i.p.) saline solution and were confined immediately to one of the compartments for 15 min (unpaired compartment). The next day, mice were administered saline solution (saline group) or alcohol (0.9 or 1.8 g/kg, 20% vol/vol solution; alcohol groups) and were confined to the other compartment drug-paired compartment) (Fig. 1a, *acquisition*). This schedule was repeated twice more (i.e., three alcohol-conditioning tests). On day 8, animals were allowed to explore the entire apparatus for 15 min (Fig. 1a, *post-acquisition test*).

Alcohol priming-induced reinstatement of alcohol CPP

Animals were conditioned with alcohol (1.8 g/kg) or saline and one day after the *post-acquisition test*, the extinction phase started with one daily extinction session for 4 d (days 9-12) during which mice received only saline injection prior confinement to the unpaired (days 9 and 10) and drug-paired compartment (days 11 and 12). On the *post-extinction test* (day 13), mice freely explored the entire CPP apparatus for 15 min. Finally, mice from each conditioning group were divided in two sub-groups with similar levels of expression and extinction of alcohol CPP (i.e. similar CPP score values calculated on *post-conditioning* and *post-extinction* tests). On the *reinstatement test* (day 14), half of the mice received a priming injection of saline (Sal/Sal and Alc/Sal groups), and the other half a non-hypnotic dose of alcohol (0.9 g/kg, Sal/Alc and Alc/Alc groups), and were immediately placed in the CPP apparatus to freely explore both compartments for 15 min.

The CPP score was calculated as time spent in the drug-paired compartment on the *post-acquisition, post-extinction* or *reinstatement test* days minus time spent in the same compartment on the *pre-acquisition day*.

Western blot analysis

An independent cohort of mice underwent the alcohol priming-induced reinstatement of CPP as described above. Sixty min following the end of the reinstatement test to allow time for protein translation following alcohol-associated behaviors (Barak et al., 2013), mice were killed by cervical dislocation and the brains removed, sectioned and dissected on ice. The NAc, DMS and DLS were isolated from a 1 mm-thick coronal section located +1.70 mm to +0.70 mm anterior to bregma. The NAc section was predominantly NAc shell but with a small fraction of the NAc core included (Laguesse et al., 2016). Tissues were homogenized in ice-cold radio immunoprecipitation assay (RIPA) buffer (in mM: 50 Tris-Cl, 5 EDTA, 120 NaCl, 1% NP-40, 0.1% deoxycholate, 0.5% SDS, protease and phosphatase inhibitors), using a cell disruptor. Protein content was determined using the BCA kit. Tissue homogenates (20 μg per sample) were resolved on NuPAGE 10% Bis-Tris gels at 100 V for 2 hrs and transferred onto nitrocellulose membranes at 30V for 2 hrs. Membranes were blocked with 5% milk-phosphate-buffered saline with 0.1% tween-20 at RT and then probed with primary antibodies overnight at 4 °C. Membranes were then washed and probed with HRP-conjugated secondary antibodies for 2 hrs at room temperature and bands were visualized using ECL. Band intensities were quantified by ImageJ (National Institutes of Health, MD, USA).

Immunohistochemistry

A separate cohort of mice underwent the alcohol priming-induced reinstatement of CPP as described above. Thirty min following the end of the reinstatement test, a time that has been previously used to study mTORC1 activation following alcohol-associated behaviors (Barak et al., 2013), mice were deeply anesthetized and then transcardially perfused with 0.01 M

phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in PBS. Brains were post-fixed in 4% (PFA) overnight and then cryoprotected in 30% sucrose for at least 3 days. Brains were then rapidly frozen and sectioned coronally into 50 µm sections using a Leica CM3050 cryostat (Leica Biosystems, Richmond, VA). Sections containing the nucleus accumbens were collected, washed in PBS, and then incubated at RT for 4 hrs in PBS containing 0.3% Triton-X for permeabilization and 5% normal donkey serum for blocking. Following PBS washes, sections were incubated in the primary antibodies rabbit anti-phospho-S6 and mouse anti-NeuN at 4°C overnight. Following washes, sections were incubated in secondary antibodies, donkey-anti rabbit IgG Alexafluor488 and donkey anti-mouse Alexafluor647, for 4 hrs at 4°C. Sections were washed and then mounted onto Fisher Superfrost glass slides. Vectashield Mounting Media was added, and slides were coverslipped. Images were acquired on the Zeiss LSM 510 Meta confocal microscope (Zeiss, Thornwood, NY) with the 20X Plan-Apochromat objective. Quantification was completed semi-automatically using Imaris, Surface module (Bitplane, South Windsor, CT) *and all counts were verified manually*. The number of NeuN+ and phospho-S6+ cells were counted within the NAc shell and core in both hemispheres and the percentage of phospho-S6- and NeuN- double positive cells over the NeuN-positive cells was calculated.

References

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