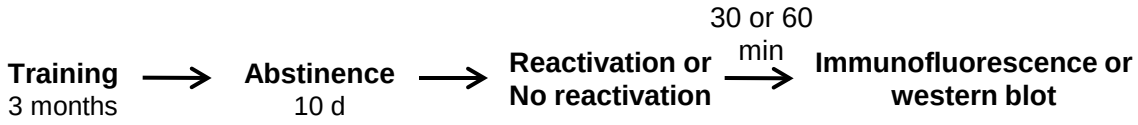
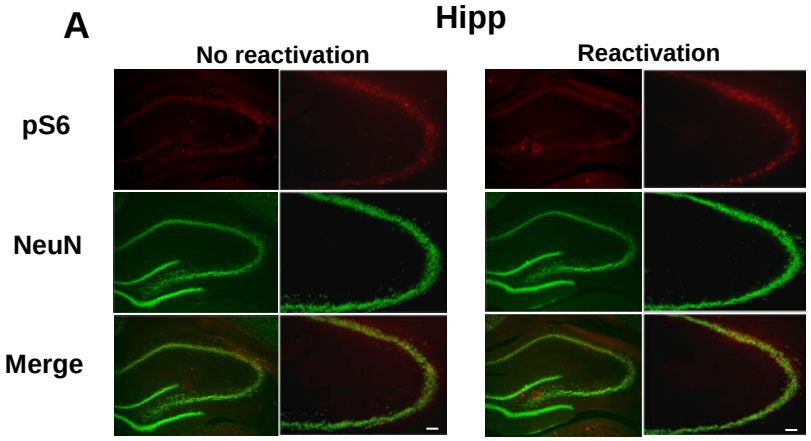
Supplementary information

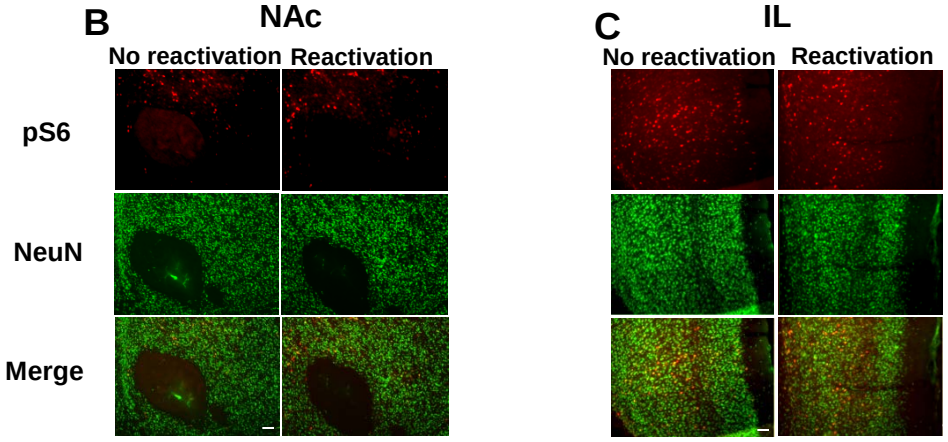
Supplemental Table 1. Lever presses (Mean ± SEM) during the 5-min reactivation session in the operant chambers. Vehicle, rapamycin or anisomycin was administered immediately after the reactivation session. Differences are not significant, p's>0.05.

Experiment		Vehicle	Rapamycin/anisomycin
Systemic rapamycin (20 mg/kg)	Immunohistochemistry	19.00 ± 5.53	-
	Western blot I	22.25 ± 5.68	-
	Western blot II	19.50 ± 3.29	20.75 ± 2.30
	Test/reacquisition	18.00 ± 2.71	16.42 ± 3.07
	Sucrose	8.07 ± 2.30	10.71 ± 2.13
Intra-CeA	Rapamycin (50 µg/side)	18.13 ± 3.46	20.38 ± 4.01
	Anisomycin (50 µg/side)	19.33 ± 2.88	17.29 ± 3.54

Barak et al., Supplemental Fig. 1

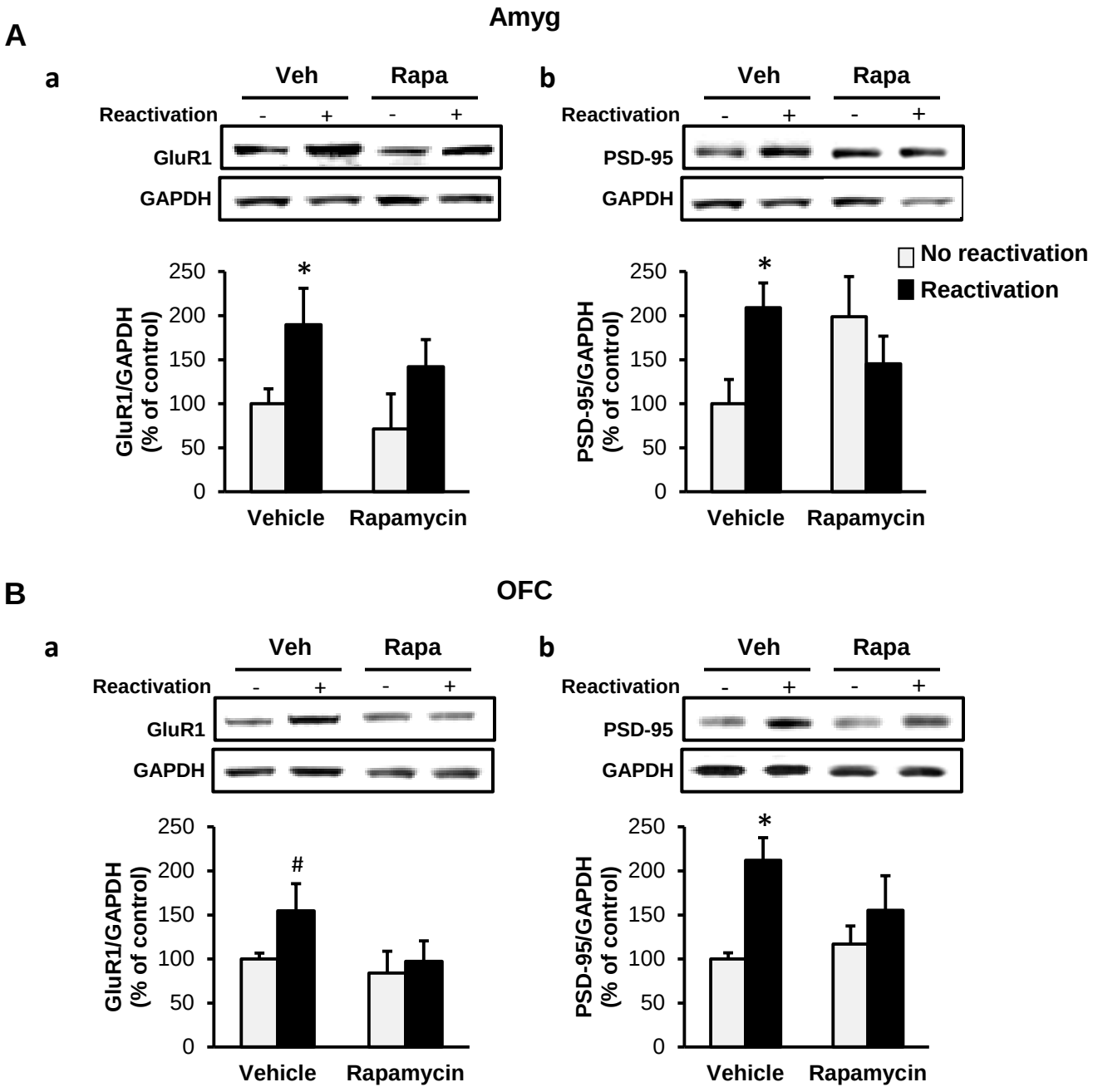






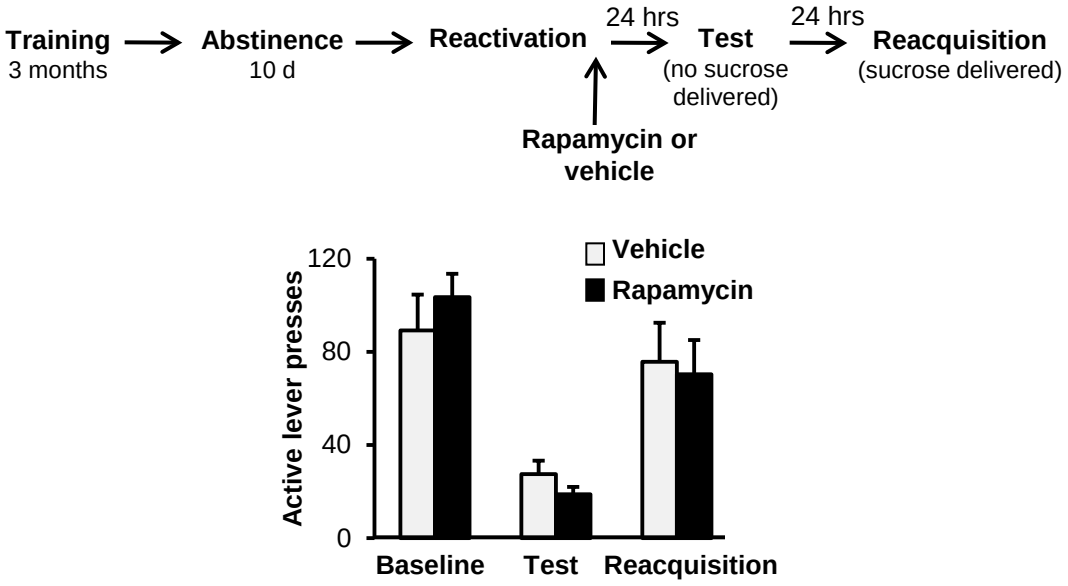
Supplemental Figure 1. The mTORC1 signaling pathway is not activated in the hippocampus, nucleus accumbens and infralimbic cortex following reactivation of alcohol-associated memories. A-C. *Immunohistochemical staining of S6 phosphorylation*. A-C. Shown is dual-channel immunofluorescence images of phosphoS6 (pS6, red), NeuN (a marker for neurons, green), and overlay (yellow), the dorsal hippocampus (A); the nucleus accumbens (NAc; B); and the infralimbic region of the medial prefrontal cortex (IL; C). Images are representative of results from 4 rats (3-4 sections/region/rat). Scale bar: 100 µm**.**

Barak et al., Supplemental Fig. 2



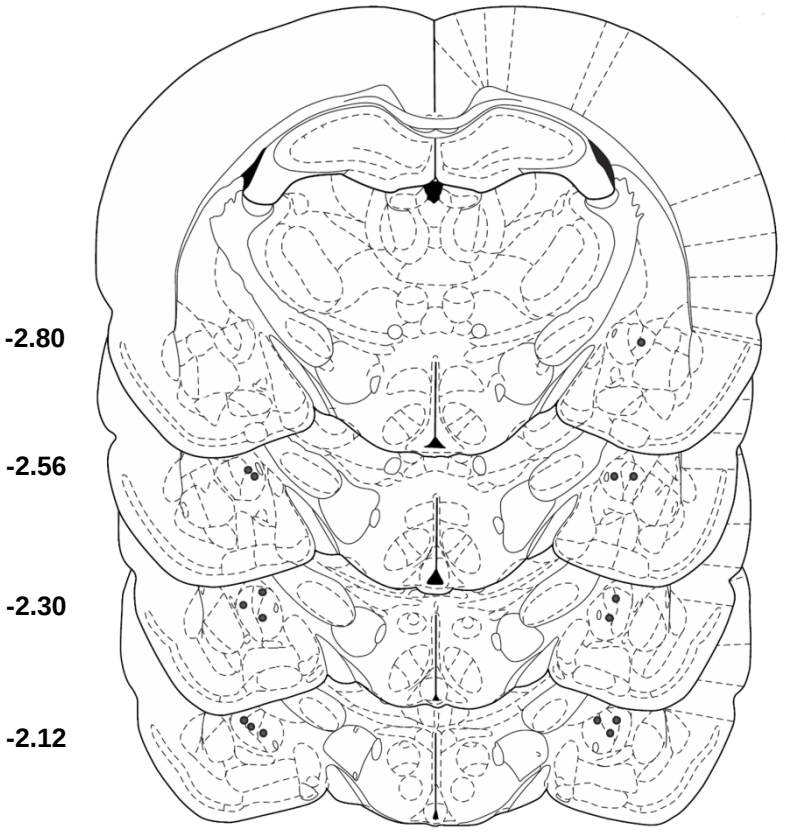
Supplemental Figure 2. Reactivation of alcohol-associated memories increases levels of synaptic proteins. *Immunoblotting of GluR1 (Aa, Ba) and PSD-95 (Ab, Bb) in the amygdala (Amyg; A) and OFC (B), 60 min after reactivation of alcohol-associated memory.* The levels of GluR1 and PSD-95 determined by western blot analysis and normalized to GAPDH. Rapamycin (20 mg/kg, i.p) was administered immediately after memory reactivation. Data are mean ± SEM and expressed as percentage of control. Aa, Ba, Bb, Two-way ANOVA; non-significant Reactivation X Treatment interaction; Ab, Reactivation X Treatment interaction [F(1, 11=4.54, p=0.05] post hoc comparisons *p<0.05 #p=0.07; n=3-4 per group).

Barak et al., Supplemental Fig. 3



Supplemental Figure 3. Inhibition of mTORC1 after reactivation of sucrose-associated memories does not affect relapse measured as instrumental responding for sucrose. Effects of rapamycin (20 mg/kg ,i.p.) given immediately after memory reactivation using presentation of context as well as a sucrose solution prime, on lever presses during test and reacquisition. Data are mean ± SEM of active lever presses before abstinence (baseline), and during retention test and reacquisition stages. (Two-way ANOVA; Stage X Treatment interaction [$F(2,26)=1.65$, $p=0.21$], n=14).

Barak et al., Supplemental Fig. 4



Supplementary Figure 4. Schematic representation of the cannulae placement in the central nucleus of the amygdala in coronal sections[3]. The locations of the cannulae tips are represented by black circles. Numbers indicate the distance relative to Bregma, in mm.

Barak et al., Supplemental Fig. 5

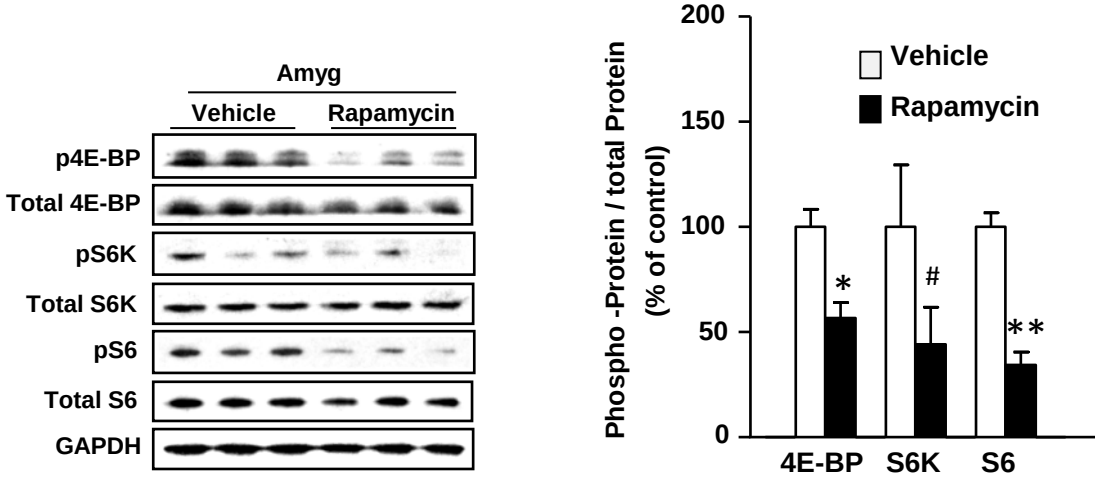
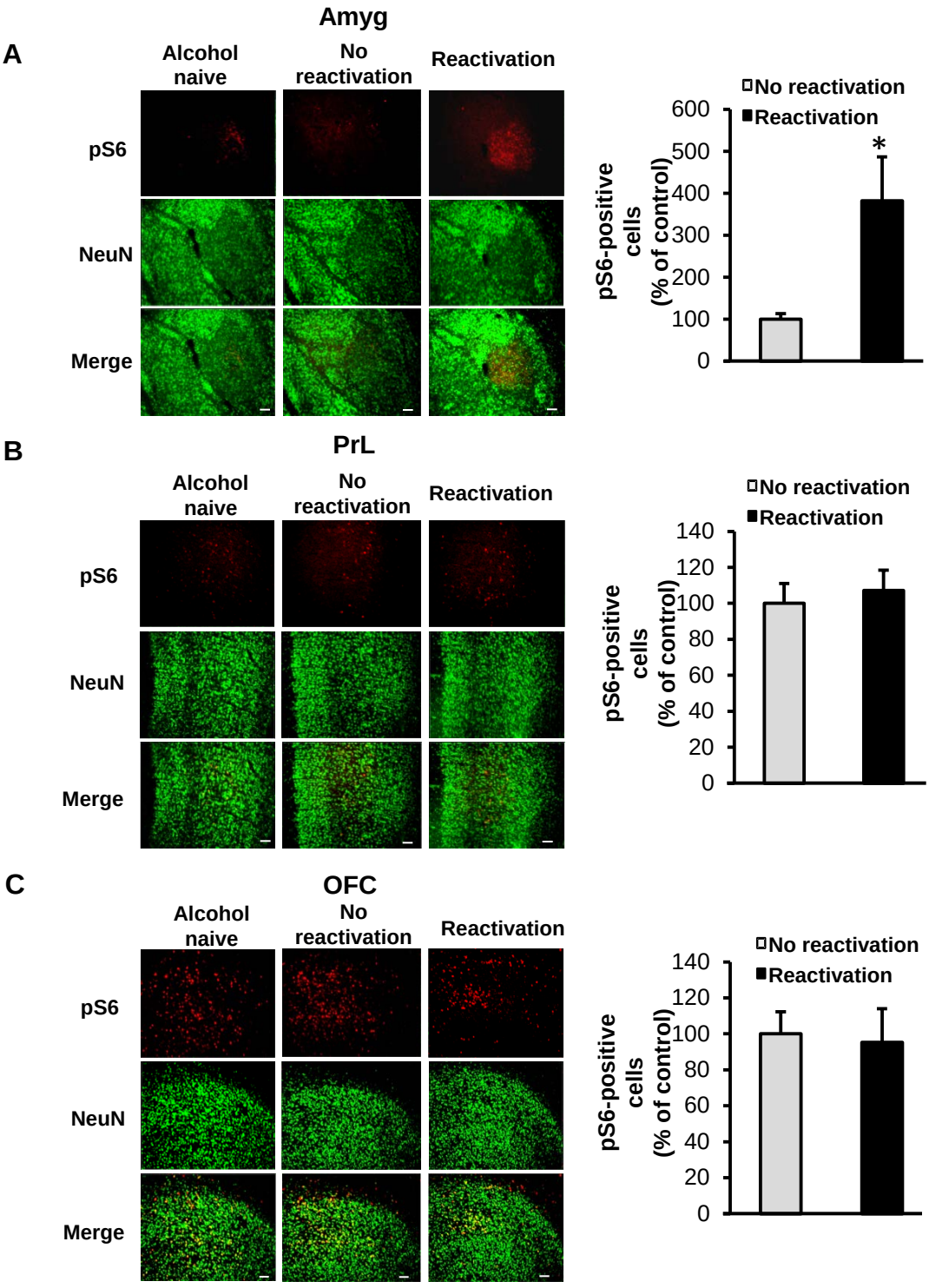


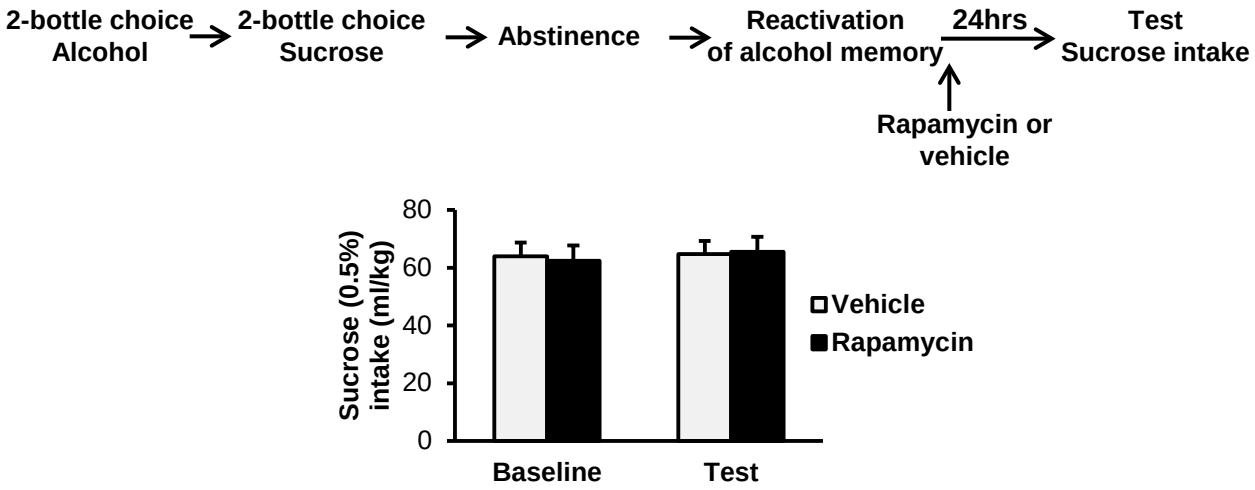
Figure 5. Infusion of rapamycin into the central nucleus of the amygdala (CeA) locally inhibits mTORC1 signaling. Rapamycin (50 µg/side) was infused unilaterally into rats' CeA; the other side was infused with vehicle. Three hrs later, Immunoreactivity of 4E-BP, S6K and S6 phosphorylation was determined by western blot analysis, normalized to the total protein level. Data are expressed as percentage of vehicle. (t's(4)>2.65, #p=0.06; *p<0.05, **p<0.01, n=3).

Barak et al., Supplemental Fig. 6



Supplemental Figure 6. The mTORC1 signaling pathway is activated in the CeA but not in the PrL or OFC following reactivation with of alcohol-associated memories odor-taste cue. A-C. Shown is dual-channel immunofluorescence images of phosphoS6 (pS6, red), NeuN (a marker for neurons, green), and overlay (yellow), the CeA (A), the PrL (B) and the OFC (C) of alcohol-naïve and alcohol-experienced rats that underwent memory reactivation by a brief exposure to the odor-taste of alcohol in the home cage (alcohol-naïve and reactivation, respectively), and of alcohol-experienced control rats that did not have a memory reactivation session (no reactivation). Images are representative of results from 4 rats (3-4 sections/region/rat). Scale bar, 100 µm. Quantification of the immunohistochemical staining was conducted by blind counting of pS6-positive cells normalized by the total area, in 3 slices per brain region from each rat. Data are mean ± SEM ($t(6)>2.67$; *$p<0.05$, $n=4$).

Barak et al., Supplemental Fig. 7



Supplemental Figure 7. Inhibition of mTORC1 after reactivation of alcohol-associated memories does not affect non-reactivated memories. Effects of rapamycin given after reactivation of alcohol-associated memories on reinstatement of sucrose intake in a 2-bottle choice procedure. Data are presented as mean ± SEM of sucrose (0.5% solution) intake (ml/kg/24 hrs) during a 24 hrs 2-bottle choice session, in rapamycin- or vehicle-treated rats before abstinence (baseline) and 24 hrs after reactivation (test; two-way ANOVA; no significant Stage X Treatment interaction $F(1,14)=0.63$, $p=0.84$; $n=8$).