Activation of nicotinic acetylcholine receptors induces potentiation and synchronization within *in vitro* hippocampal networks

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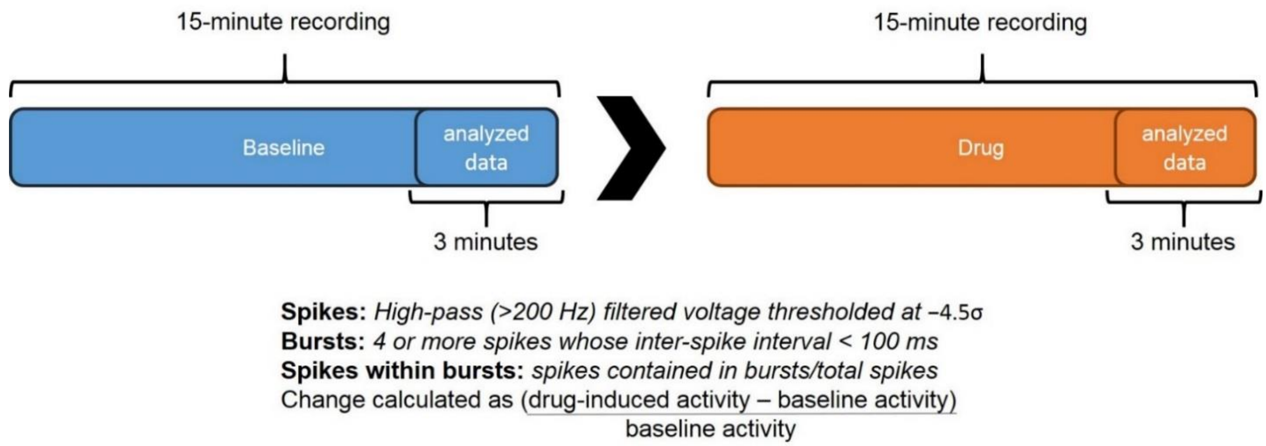

Figure S1. Schematic of the experimental design and analysis of spiking data.

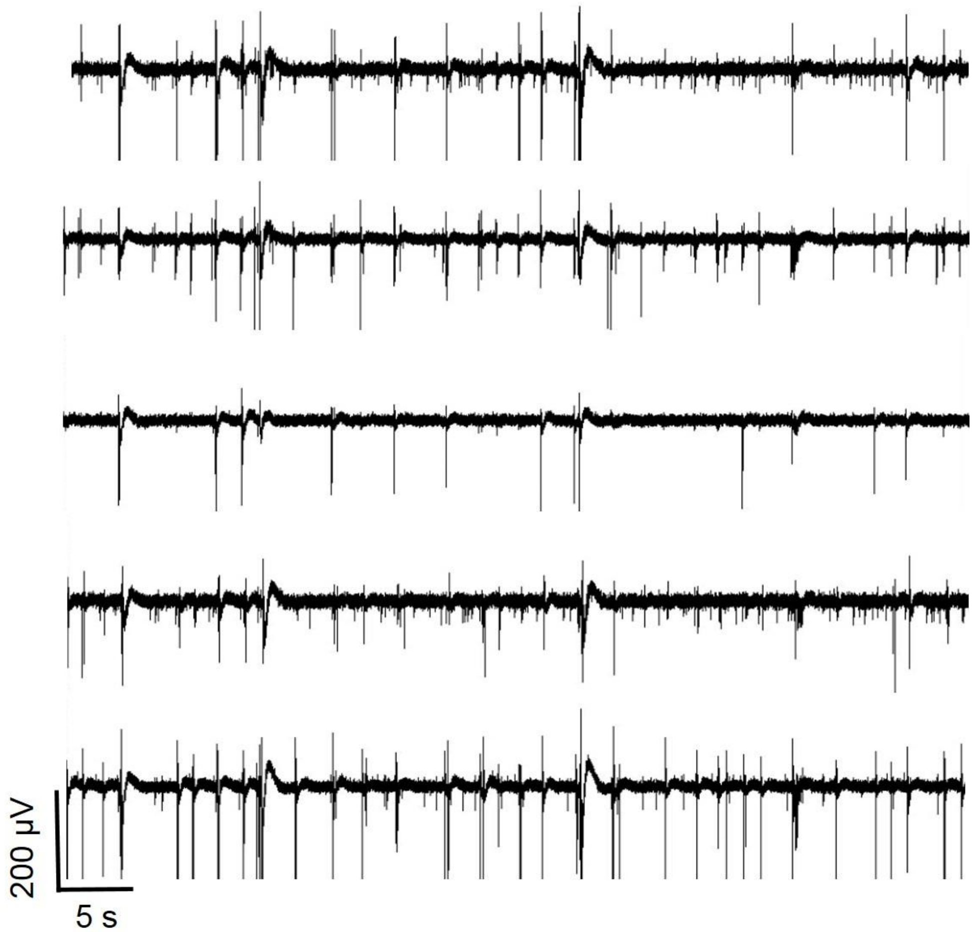

Figure S2. Spontaneous activity from primary cultured DIV14 rat hippocampal neural networks depicting 1-minute traces of raw baseline network activity from 5 representative electrodes (voltage display cutoff at 200 μV).

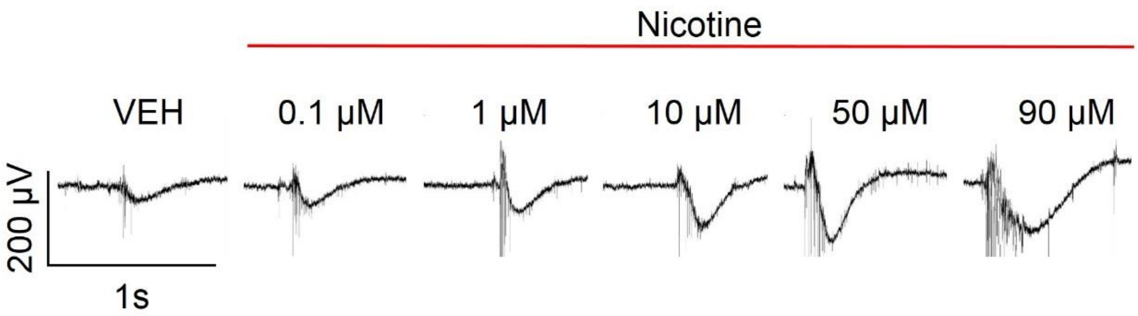

Figure S3. Single representative electrode 1 second traces of raw signal from nicotine or vehicle-treated DIV14 primary cultured rat hippocampal neural networks (voltage display cutoff at 200 μV).

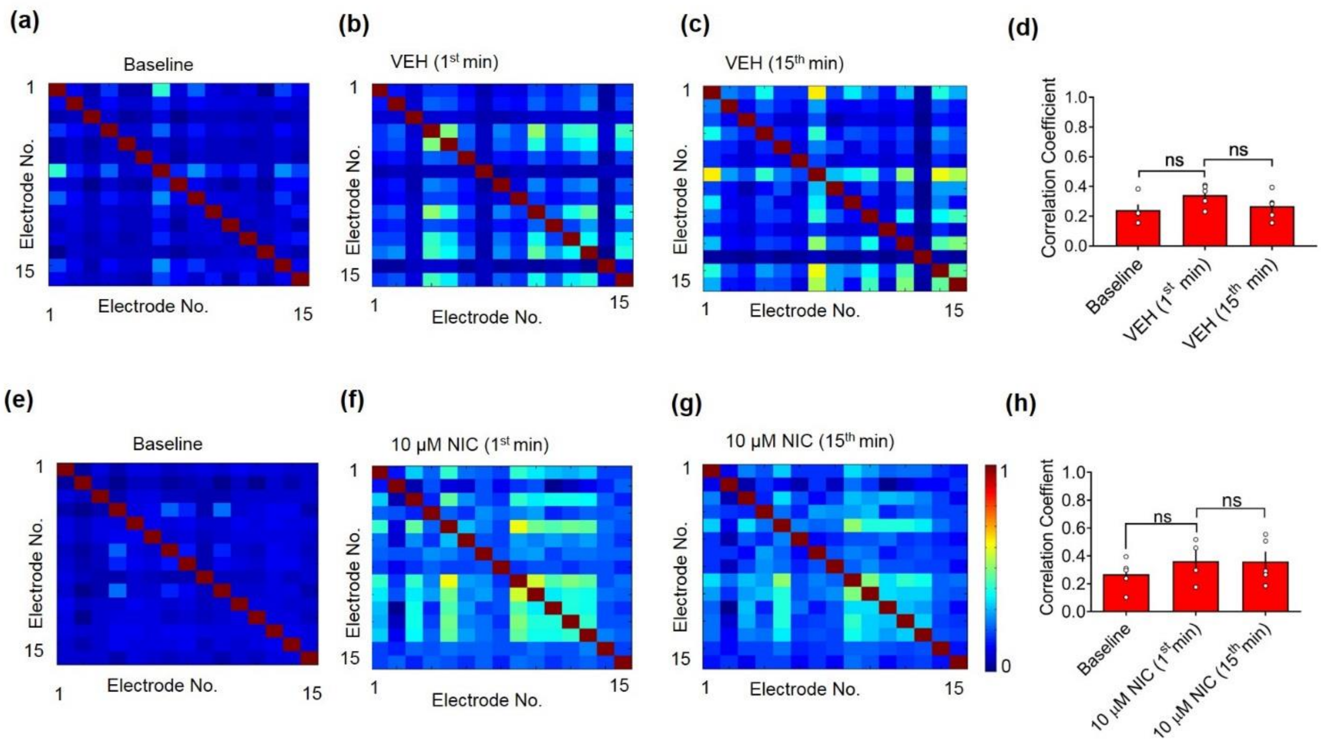

Figure S4. Vehicle and 10 μM nicotine do not induce network synchrony. (a-c) Representative spatial maps of correlation coefficients between active electrodes of the network at (a) baseline, (b) 1 minute, and (c) 15 minutes after applying vehicle. (d) Quantification of a-c. (e-g) Representative spatial maps of correlation coefficients between active electrodes of the network at (e) baseline, (f) 1 minute, and (g) 15 minutes after applying 10 μM nicotine. (h) Quantification of e-g. Statistical significance was assessed by a repeated-measures ANOVA, followed by a Tukey's post hoc correction (ns = not significant).

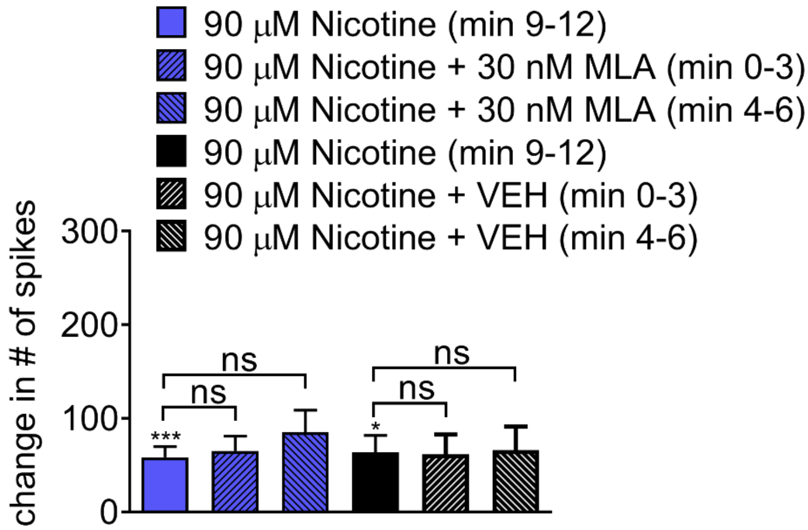

Figure S5. Steady-state activity of α7 nAChRs does not contribute to the long-lasting effects of nicotine on spiking. Effects of 90 μM nicotine on spikes (before and during co-application with the α7 nAChR antagonist, MLA [blue] or vehicle [black]). 90 μM nicotine increases in spiking (min. 9-12). When applied for 6 minutes at the 13th minute of stimulation with 90 μM nicotine, both MLA [blue] and vehicle [black] do not significantly change the spiking. Baseline spiking values presented as mean ± SEM: MLA-treated group (0.584829± 0.1309, n=5); vehicle-treated group (0.637065± 0.140104, n=3). Statistical significance of the treatment with 90 μM nicotine was calculated via a one-sample t-test. Statistical difference between the effect of nicotine before or after the application of MLA or vehicle was assessed by a repeated-measures ANOVA, followed by a Tukey's post hoc. (*$p<0.05$ and ***$p<0.001$). Data are normalized to baseline.

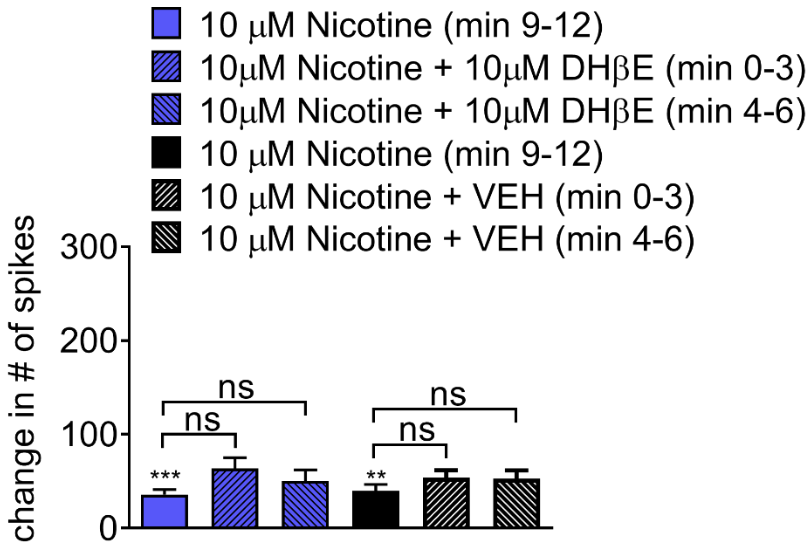

Figure S6. Steady-state of α4β2 nAChRs does not contribute to the long-lasting effects of nicotine on spiking. Effects of 10 μM nicotine on spiking (before and during co-application with the α4β2 nAChR antagonist, DHβE [blue] or vehicle [black]). 10 μM nicotine increases in spiking (min. 9-12). When applied for 6 minutes at the 13th minute of stimulation with 10 μM nicotine, both DHβE [blue] and vehicle [black] do not significantly change the spiking. Baseline spiking values presented as mean ± SEM: DHβE -treated group (0.359944± 0.045189, n=4); vehicle-treated group (0.371782± 0.074619, n=3). Statistical significance of the treatment with 10 μM nicotine was calculated via a one-sample t-test. Statistical difference between the effect of nicotine before or after the application of DHβE or vehicle was assessed by a repeated-measures ANOVA, followed by a Tukey's post hoc. (**p<0.01 ***p<0.001). Data are normalized to baseline.

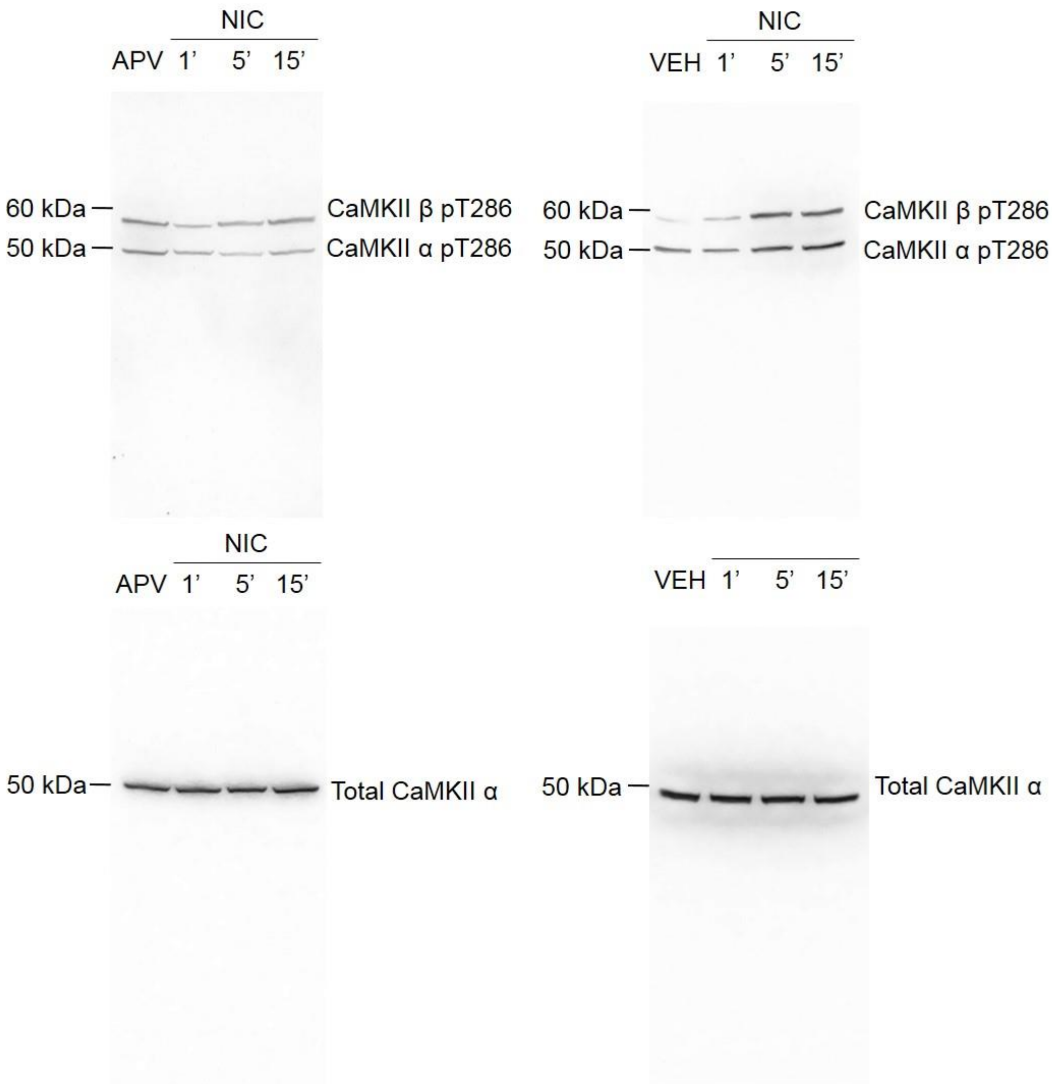

Figure S7. Full blots from Figure 6.

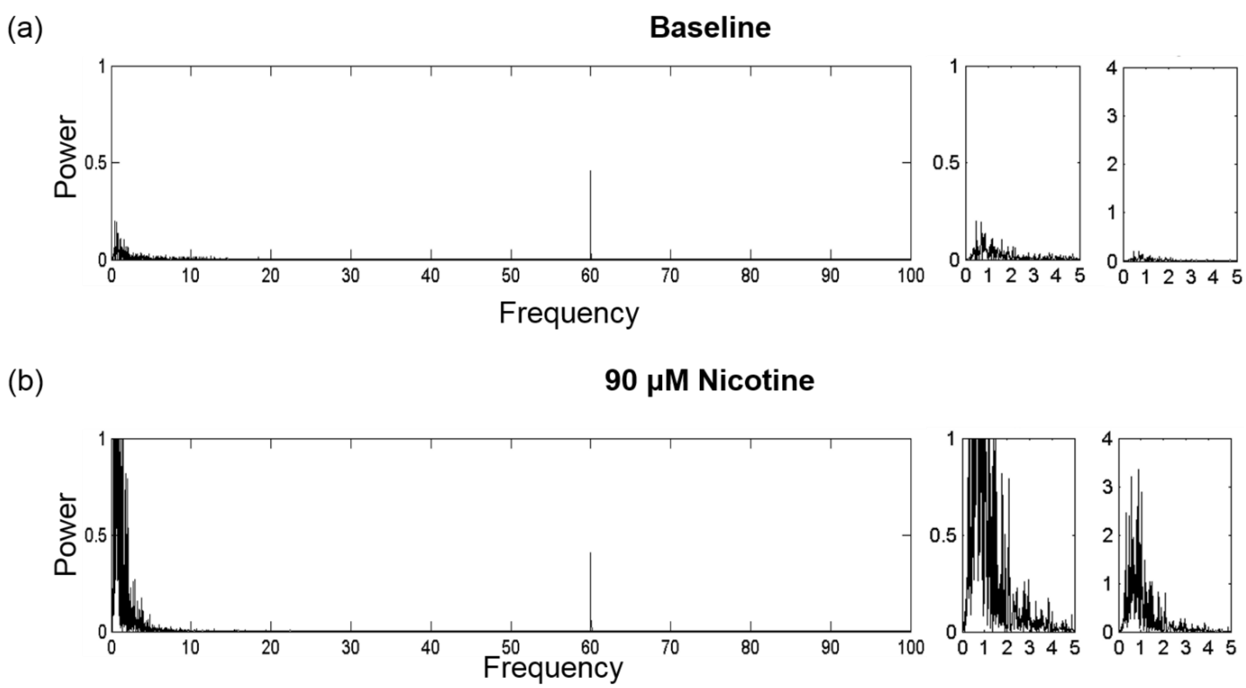

Figure S8. Representative power spectrum plots of 1 KHz down-sampled data. Nicotine strengthens but does not alter the frequency of preexisting network oscillations. The power of preexisting oscillations (a) was increased by 90 μM nicotine (b) in the range between 0-5 Hz (expanded view of this range shown in graphs to the right). Note that the 60 Hz peak is power-line noise and is present in both baseline (a) and post-nicotine treatment (b).

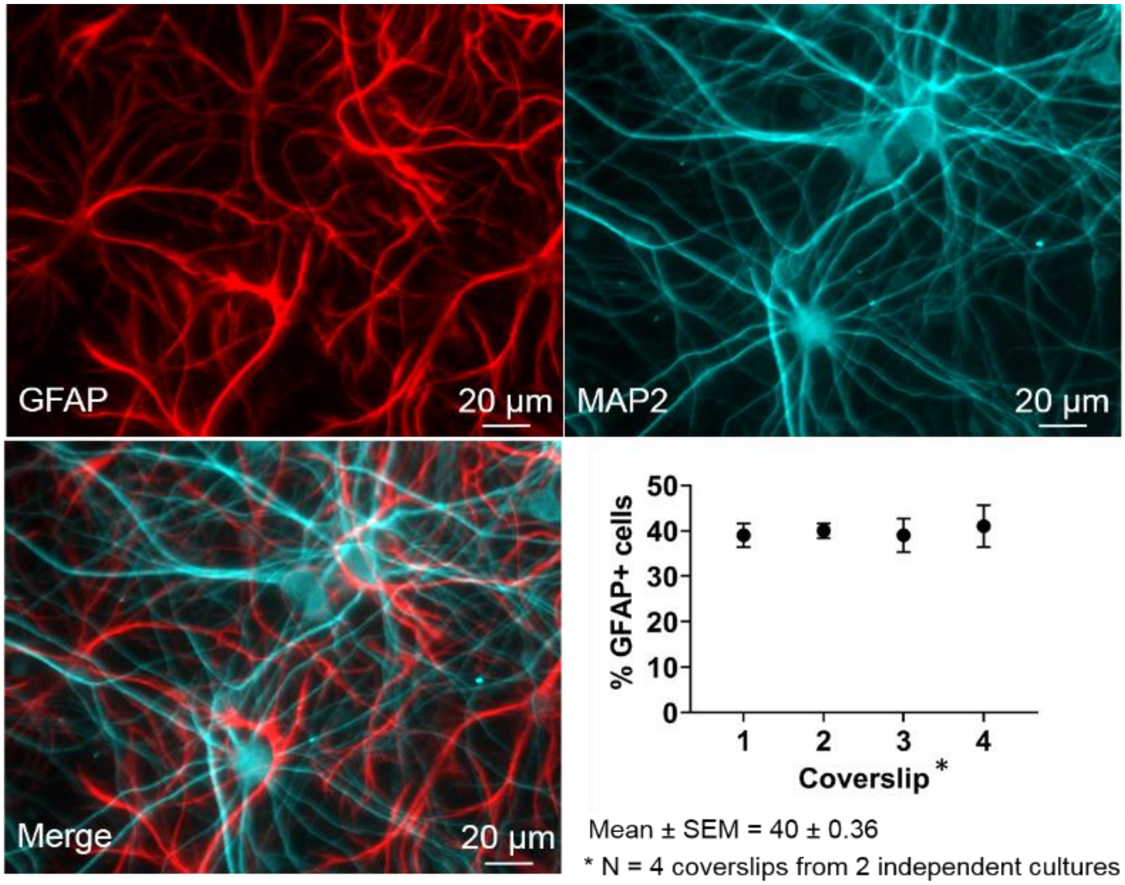

Figure S9. Astroglial quantification in primary hippocampal cultures. Cultures (DIV14) grown on glass coverslips were immunostained using anti-GFAP (red, upper left panel) and anti-MAP2 (cyan, upper right panel) antibodies, with merged images shown as indicated (lower left panel). The percentage of $GFAP^+$ cells ($100*GFAP^+ / (GFAP^+ + MAP2^+)$) is quantified at bottom right. Mean ± SEM = 40 ± 0.36; N=4 coverslips from 2 independent cultures.

Baseline values for Figures 2, 3, and 5.

Fig. 2. Baseline values for **nicotine** treated cultures reported as mean ± SEM (N=5 for each treatment). *Spikes*: nicotine 0.1 µM {23120 ± 6564}, 1 µM {11383 ± 993}, 10 µM {28681 ± 5244}, 50 µM {11596 ± 2360}, 90 µM {21909 ± 4741}, VEH {9555 ± 1966}; *Bursts*: nicotine 0.1 µM {1296 ± 386}, 1 µM {737 ± 84}, 10 µM {1767 ± 308}, 50 µM {768 ± 188}, 90 µM {1245 ± 290}, VEH {646 ± 97}; *Spikes within bursts (as a fraction of the total number of spikes)*: nicotine 0.1 µM {0.51 ± 0.09}, 1 µM {0.52 ± 0.04}, 10 µM {0.60 ± 0.05}, 50 µM {0.54 ± 0.06}, 90 µM {0.43 ± 0.02}, VEH {0.59 ± 0.03}.

Fig. 3 Baseline values for **MLA** treated cultures reported as mean ± SEM (N=5 for each treatment), *Spikes*: VEH {9555 ± 1966}, 30 nM MLA {10773 ± 1613}, 30 nM MLA + 90 µM nicotine {10773 ± 1613}, 90 µM nicotine {21909 ± 4741}; *Bursts*: VEH {646 ± 97}, 30 nM MLA {530 ± 125}, 30 nM MLA + 90 µM nicotine {530 ± 125}, 90 µM nicotine {1245 ± 290}; *Spikes within bursts (as a fraction of the total number of spikes)*: VEH {0.59 ± 0.03}, 30nM MLA {0.50 ± 0.07}, 30 nM MLA + 90 µM nicotine {0.50 ± 0.07}, 90 µM nicotine {0.43 ± 0.02}. Baseline values for **SAZ-A** treated cultures reported as mean ± SEM (N=5 for each treatment), *Spikes*: VEH {9555 ± 1966}, 1 µM SAZ-A {22300 ± 5658}, 1 µM SAZ-A + 90 µM nicotine {22300 ± 5658}, 90 µM nicotine {21909 ± 4741}; *Bursts*: VEH {646 ± 97}, 1 µM SAZ-A {1199 ± 326}, 1 µM SAZ-A + 90 µM nicotine {1199 ± 326}, 90 µM nicotine {1245 ± 290}; *Spikes within bursts (as a fraction of the total number of spikes)*: VEH {0.59 ± 0.03}, 1µM SAZ-A {0.49 ± 0.06} , 1 µM SAZ-A + 90 µM nicotine {0.49 ± 0.06}, 90 µM nicotine {0.43 ± 0.02}. Baseline values for **AT-1001** treated cultures reported as mean ± SEM (N=5 for each treatment), *Spikes*: VEH {9555 ± 1966}, 20 µM AT-1001 {14338 ± 1496}, 20 µM AT-1001 + 90 µM nicotine {14338 ± 1496}, 90 µM nicotine {21909 ± 4741}; *Bursts*: VEH {646 ± 97}, 20 µM AT-1001 {1065 ± 148}, 20 µM AT-1001 + 90 µM nicotine {1065 ± 148}, 90 µM nicotine {1245 ± 290}; *Spikes within bursts (as a fraction of the total number of spikes)*: VEH {0.59 ± 0.03}, 20 µM AT-1001 {0.52 ± 0.04}, 20 µM AT-1001 + 90 µM nicotine {0.52 ± 0.04}, 90 µM nicotine {0.43 ± 0.02}.

Fig. 5 Baseline values for **MK-801** treated cultures reported as mean ± SEM (N=4 for each treatment), *Spikes*: VEH {9555 ± 1966}, 10 µM MK-801 {7336 ± 704}, 10 µM MK-801 + 90 µM nicotine {7336 ± 704}, 90 µM nicotine {21909 ± 4741}; *Bursts*: VEH {646 ± 97}, 10 µM MK-801 {376 ± 69}, 10 µM MK-801 + 90 µM nicotine {376 ± 69}, 90 µM nicotine {1245 ± 290}; *Spikes within bursts (as a fraction of the total number of spikes)*: VEH {0.59 ± 0.03}, 10 µM MK-801 {0.37 ± 0.07}, 10 µM MK-801 + 90 µM nicotine {0.37 ± 0.07}, 90 µM nicotine {0.43 ± 0.02}. Baseline values for **MPEP + 3-MATIDA** treated cultures reported as mean ± SEM (N=4 for each treatment), *Spikes*: VEH {9555 ± 1966}, 1 µM MPEP & 100 µM 3-MATIDA {12100 ± 11529} , 1 µM MPEP & 100 µM 3-MATIDA + 90 µM nicotine {12100 ± 11529}, 90 µM nicotine {21909 ± 4741}; *Bursts*: VEH {646 ± 97}, 1 µM MPEP & 100 µM 3-MATIDA {778 ± 132}, 1 µM MPEP & 100 µM 3-MATIDA + 90 µM nicotine {778 ± 132}, 90 µM nicotine {1245 ± 290}; *Spikes within bursts (as a fraction of the total number of spikes)*: VEH {0.59 ± 0.03}, 1 µM MPEP & 100 µM 3-MATIDA {0.56 ± 0.02}, 1 µM MPEP & 100 µM 3-MATIDA + 90 µM nicotine {0.56 ± 0.02}, 90 µM nicotine {0.43 ± 0.02}.