Supplemental Fig. S1. Dose response relationships for forskolin- and 8-Br-cAMP-induced CREB-dependent gene expression (4xCRE luciferase reporter activity). The inactive forskolin analog 1,9-dideoxy-forskolin (dd-forskolin, 30 µM) did not stimulate 4xCRE transcription. Where indicated, 10 µM H89 was added in the presence of 10 µM forskolin. Bars indicate mean ± sem for 3–4 experiments.
Supplemental Fig. S2. (a) Time course of stimulation of *c-fos* and *BDNF* (exons I and IV) mRNA expression by forskolin (10 μM) relative to unstimulated cultures (dotted line). *c-fos* was stimulated 7.5-fold at one hour, 6-fold at 3 hrs and 5-fold at 24 hrs. In contrast, BDNF exons I and IV RNAs were unchanged at 1 hour; exon I was stimulated by 3-fold following stimulation durations of 3–24 hrs, whereas exon IV was substantially stimulated (3-fold) only at 24 hrs. ***, and **, significantly different from unstimulated mRNA expression p<0.001 and 0.005 (n = 10, 11 and 4 at 1, 3 and 24 hrs, respectively). In Fig. 6, *c-fos* and *BDNF* mRNAs were measured at 24 hours to produce substantial stimulation of all three RNAs at a single time point. (b) Stimulation of *c-fos* mRNA expression by forskolin at 1 hour was inhibited by ifenprodil (10 μM); Bars show means ± sem; ***, significantly different from forskolin-stimulated expression p<0.001.
Supplemental Fig. S3. Potentiation of forskolin response by trans-PDC. Like TBOA (Fig. 7), the EAA uptake inhibitor, trans-PDC (30 µM), potentiated the response to forskolin (1 µM) or by 8-Br-cAMP (1 mM). *** significantly different from responses to forskolin or 8-Br-cAMP in the absence of trans-PDC (p<0.001). Trans-PDC alone (10 µM) did not stimulate 4xCRE activity (data not shown).
Supplemental Fig. S4. Glutamate scavenger does not affect cAMP-induced 4xCRE transcription. (a) Glutamate-pyruvate dehydrogenase (GPT) scavenging system degrades glutamate. In the presence of pyruvate, GPT converts L-glutamate to α-ketoglutarate + L-alanine. Glutamate samples (1, 10 or 100 nmoles) were incubated for 1 h at 37°C with pyruvate (10 mM) in the absence or presence of GPT (5 U/ml), then inactivated by heating to 95°C for 10 min. NADPH fluorescence was measured as described by Dixon and Hokin (Proc Natl Acad Sci USA 94:4757-4760, 1997) following addition of glutamate dehydrogenase, which catalyzes the conversion of L-glutamate + NAPD to 2-oxoglutarate + NADPH. GPT + pyruvate completely degraded the added glutamate. 1, 10 and 100 nmoles glutamate correspond to concentrations of 2, 20 and 200 µM in the neuron cultures. (b) Glutamate scavenger failed to inhibit forskolin-induced 4xCRE transcription. Addition of pyruvate (10 mM) plus GPT (5 U/ml) did not affect forskolin-induced 4xCRE transcription, but abolished the response to added glutamate (100 µM). Bars show means ± sem; ***, significantly different from cells exposed to glutamate but not GPT (p<0.001); ns, not significantly different from cells exposed to forskolin but not GPT.
**Supplemental Fig. S5.** Astrocytes do not require activation of NMDARs for cAMP-induced induction of 4xCRE transcription. Forskolin (50 µM) stimulated 4xCRE transcription in primary astrocyte cultures; this response was abolished by H89 (10 µM), but, in contrast to neurons (Fig. 3), was not affected by AP5 (100 µM). Bars show means ± sem; ***, significantly different from forskolin alone (p<0.001).
Supplemental Fig. S6. (a) L-type Ca\(^{2+}\) channel antagonists do not block forskolin-induced aspartate release into the medium. Cultures were stimulated with forskolin in the presence or absence of either nifedipine or verapamil for 30 min and the medium was analyzed for aspartate by HPLC as in Materials and Method and shown in Fig. 9. (b) Block of L-type Ca\(^{2+}\) channels does not affect stimulation of CREB-dependent gene transcription by threshold concentrations of aspartate. Neuron cultures were stimulated with the indicated concentrations of L-aspartate and 4xCRE luciferase activity was analyzed as described in Materials and Methods. 4xCRE expression in untreated cultures = 1.0. Bars show means ± sem; *, significantly different from untreated, p<0.05.