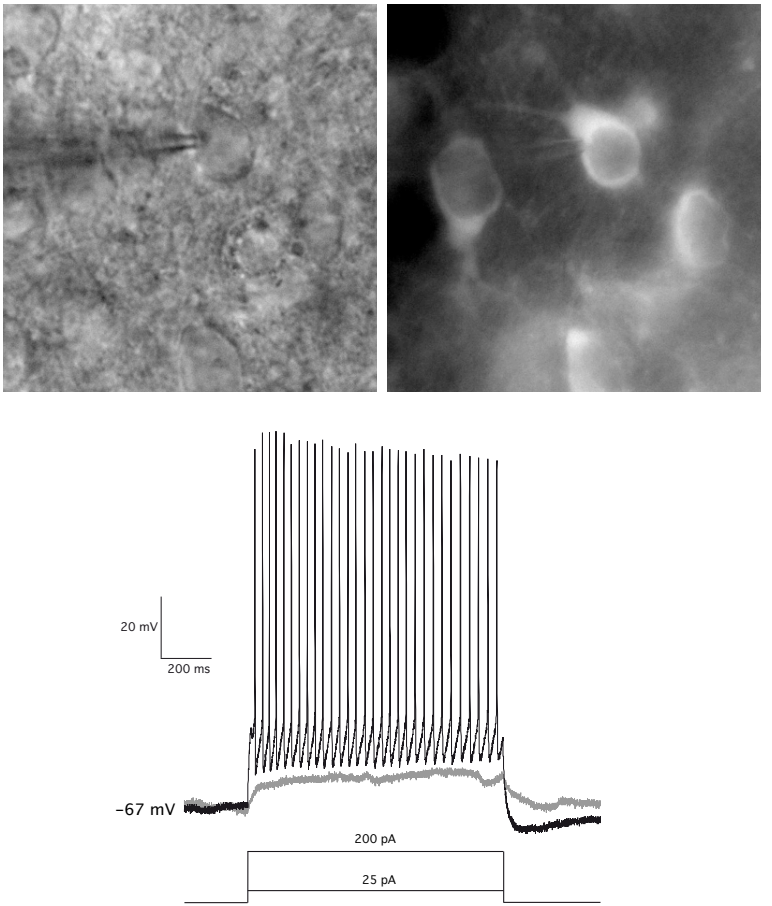
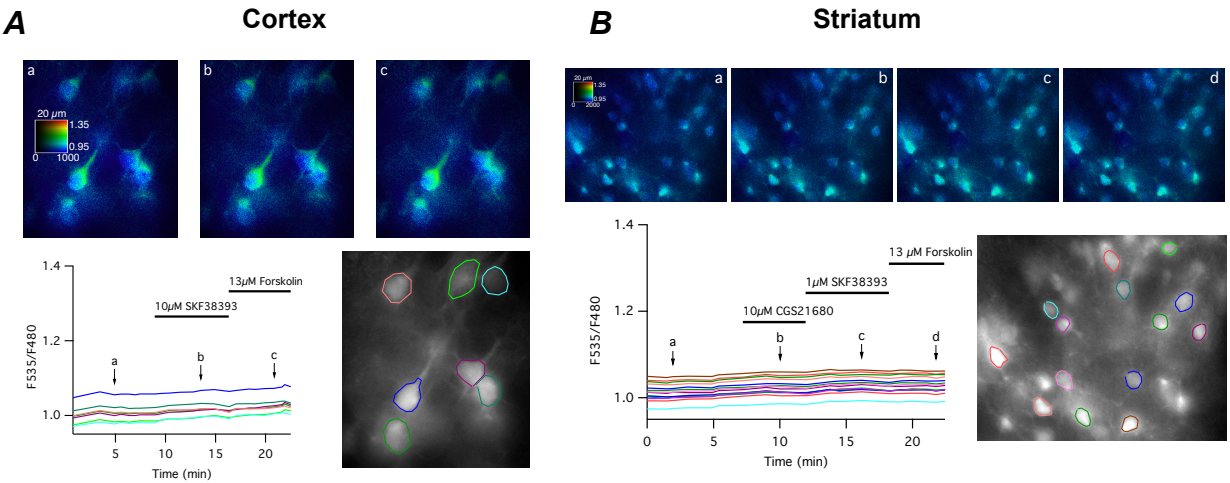


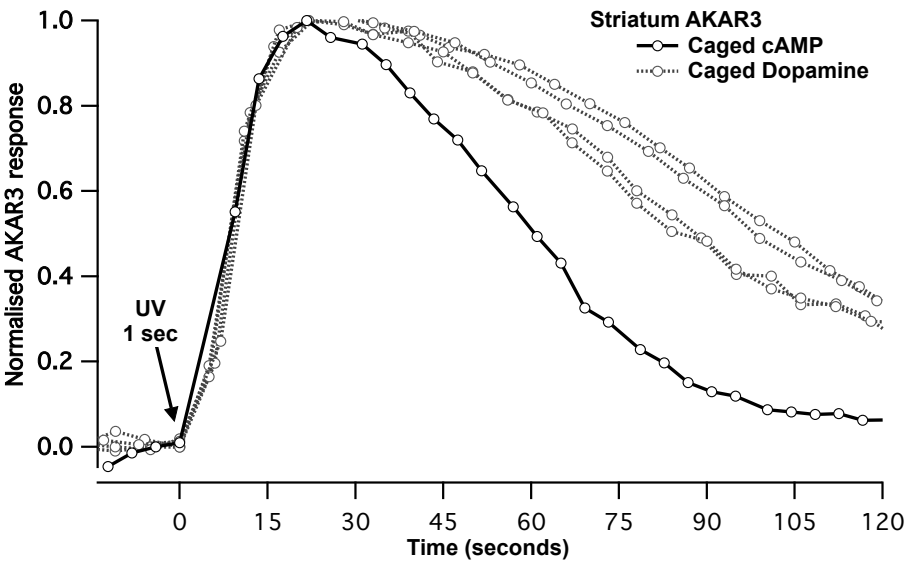
Supplementary figure 1: Neurons expressing the biosensor exhibit normal electrophysiological properties

A neuron infected by the sindbis vector for Epac1-camps was patch-clamped with a potassium methane sulfonate pipette solution. Top left: the neuron imaged in transmitted infra-red light. Top right: the same field showing the fluorescence of the biosensor. Traces below show the voltage responses to current injection in current-clamp mode (25 pA and 200 pA) in the same neuron. The input resistances was monitored in voltage-clamp mode and was R_{in}=437 MΩ ± 62 MΩ, n=4 for transfected cells and Rin=350 MΩ n=1 for a non-transfected cell.



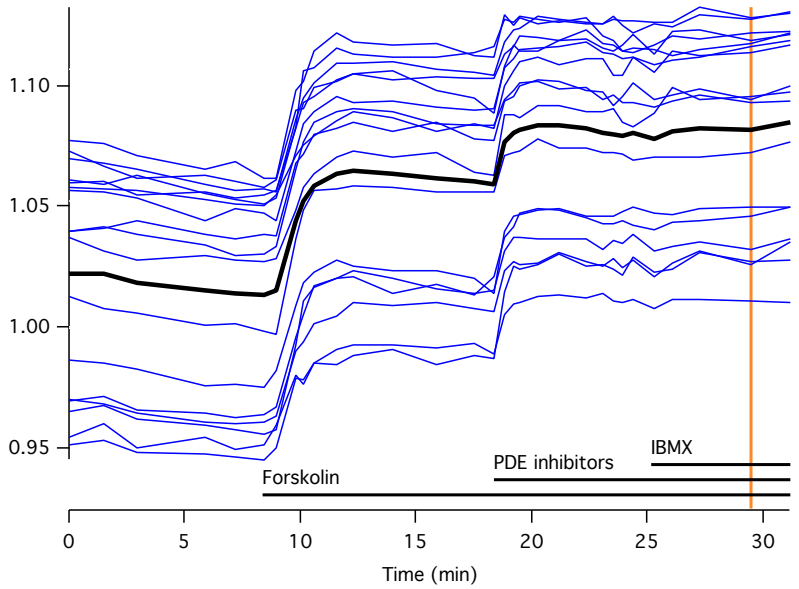
Supplementary figure 2: No response was detected when the phosphorylation site in AKAR3 was mutated.

The threonine 391 residue, which is phosphorylated by PKA, was replaced with an alanine residue. When this mutant biosensor was expressed in cortical (***A***) or striatal (***B***) neurones. No response to SKF38393, CGS21680 or forskolin was detected by wide-field imaging.



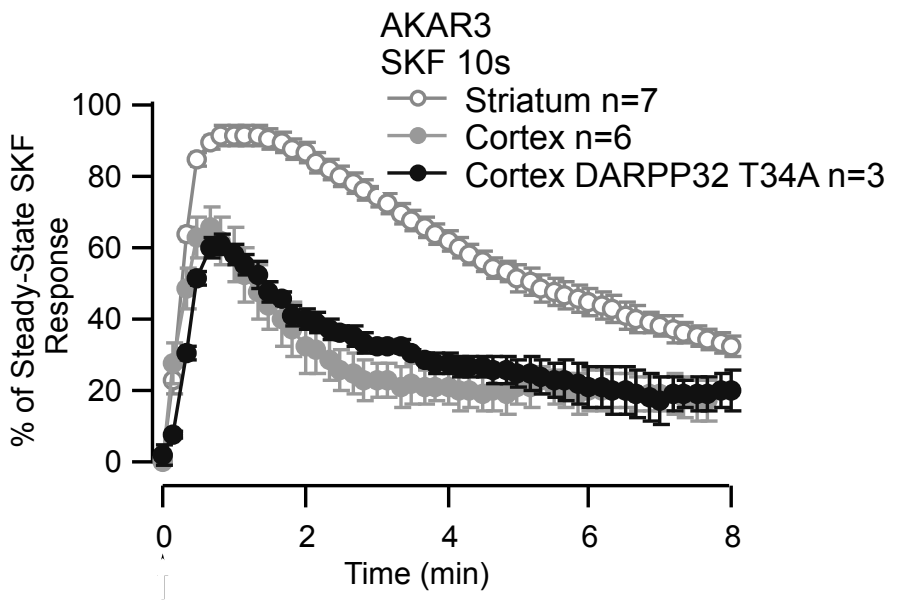
Supplementary figure 3: Temporal resolution of AKAR3 measurements

The experiment was performed using the same experimental conditions as Fig. 8. NPEC-Dopamine (caged dopamine) was applied in the bath at 5 μM concentration. DMNB-cAMP (caged cAMP) was applied in the bath at 10 μM concentration. Sampling rate was 0.2 Hz.



Supplementary figure 4: the effect of IBMX does not involve a non-specific inhibition of adenosine receptors

We inhibited PDE1 and PDE5 (zaprinast, 50 µM), PDE2 (EHNA, 100 nM) PDE3 (Cilostamide, 1µM) PDE4 (rolipram, 100nM), PDE7 (Brl50481, 1µM) and PDE10 (PQ10, 100nM). Addition of this cocktail of PDE inhibitors to forskolin induced a strong increase in the cAMP levels (n=5). Addition of IBMX did not produce any further increase.



Supplementary Figure 5: In the cortex, the T34A mutation in DARPP-32 does not affect the kinetics of the response to transient SKF38393 stimulation.

The experiment was performed using the same experimental conditions as Fig. 7.