

Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: Time-lapse confocal ratiometric images of the single N1E-115 cells co-transfected with 5-HT₄ R together with FRET-based biosensor Raichu-RhoA. After 5 min imaging under control conditions, 5-HT was added to the bath solution (indicated by the red spot) and cells were imaged for the next 15 min. Increase in the YPet/mTurquoise ratio is equivalent to an increase in the RhoA activity.

File Name: Supplementary Movie 2

Description: Time-lapse confocal ratiometric images of one filopodia from N1E-115 cells cotransfected with 5-HT₄ R together with FRET-based biosensor Raichu-RhoA. After 5 min imaging under control conditions, 5-HT was added to the bath solution (indicated by the red spot) and cells were imaged for the next 15 min. Increase in the YPet/mTurquoise ratio is equivalent to an increase in the RhoA activity.

File Name: Supplementary Movie 3

Description: Time-lapse confocal images of defined spines in the hippocampal neurons cotransfected with FRET-based biosensor RaichuRhoA (left) and LifeAct-mRuby (right). Images were acquired every 2.5 min. After 7.5 min imaging under control conditions (-7.5 min to 0 min), BIMU8 was added to the bath solution and cells were imaged for the further 10 min. Increase in the YPet/mTurquoise ratio is equivalent to an increase in the RhoA activity in a single spine. Increased fluorescence intensity for LifeActmRuby indicates the F-actin accumulation in the same spines.

File Name: Supplementary Movie 4

Description: 3D reconstruction of dendrites in hippocampal neurons transfected with FRET-based biosensor Raichu-RhoA acquired before and 15 min after treatment with vehicle. Images also show the RhoA activity within the defined spine (color-coded, as indicated on the bottom).

File Name: Supplementary Movie 5

Description: 3D reconstruction of dendrites in hippocampal neurons transfected with FRET-based biosensor Raichu-RhoA acquired before and 15 min after treatment with BIMU8. Images show also the RhoA activity within the defined spine (color-coded, as indicated on the bottom).

File Name: Supplementary Data 1

Description: Table containing all source data underlying the graphs and charts presented in the main figures.

