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# Supplementary Materials for

## A transgenic mouse for imaging activity-dependent dynamics of endogenous Arc mRNA in live neurons

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**
(available at advances.sciencemag.org/cgi/content/full/4/6/eaar3448/DC1)

## Supplementary Materials and Methods

### qRT-PCR

Hippocampal neurons from $Arc^{P/P}$ and WT mice were grown at a density of 75,000 cells/chamber of each Mattek dish. Neurons at DIV 16-18 were stimulated with bicucuclline (50 μM) for 15 mins, followed by washout, and DRB (0.45 μM), an inhibitor of transcription elongation, was added. Total RNA was extracted from neurons at different time points of DRB treatment using TriZol reagent (Ambion) and then treated with DNase for 30 min (Turbo DNAse kit, Ambion) to remove any genomic DNA. cDNA library was prepared from total mRNA using the Superscript IV Reverse Transcriptase kit (Invitrogen). Arc primers were designed against the coding sequence of Arc (Forward primer: 5'- CACTCTCCCGTGAAGCCATT-3', and reverse primer: 5'-GGTGCCCACCACATACTGAA-3'), which generated a PCR product of 150 bp for both wild-type and Arc-PBS mRNA. SYBR Green was used for a 2-step qRT-PCR reaction, run on 7900HT Real-time PCR system (Applied Biosystems). Samples were run in duplicates, and analyzed using Quant Studio Real-Time PCR software. Arc mRNA levels were analyzed by ΔCt method, and normalized to GAPDH levels. Relative mRNA levels were measured, and 15 min after DRB treatment was considered 100%, since the drug acted 10-15 min after addition to cultures. Values were fitted to a single exponential decay function to calculate $t_{1/2}$.

### Western blotting

Brain tissue extracts were prepared using T-PER tissue protein extraction reagent (Thermo scientific) containing 1× protease inhibitor (Roche). Three 5-week-old female wild-type and Arc-PBS mice were sacrificed according to IACUC guidelines. 32 μg of protein was separated on 4-12% Bis-Tris polyacrylamide precast gels in MES-SDS running buffer and transferred to nitrocellulose membranes by using the Mini Blot Module following the manufacturer's instructions (Thermo scientific). The following antibodies were used: anti-Arc (1:200, sc-17839, Santa Cruz Biotechnology) and anti-

GAPDH (1:100,000, G9545, Sigma) as primary antibodies, and anti-rabbit IgG conjugated to HRP (1:5,000, SA002, GenDEPOT), and anti-mouse IgG conjugated to HRP (1:5,000, SA001, GenDEPOT) as secondary antibodies. Pierce ECL western blotting substrate (Thermo scientific) was used for HRP detection. Western blots were imaged by LAS 4000 (GE Healthcare Life Sciences). The images were analyzed by Image Studio Lite Ver 5.2 (LI-COR Biosciences).

**Image Analysis for $Ca^{2+}$ activity**

$Ca^{2+}$ plots were obtained by using FluoroSNNAP program (*61*) and custom-made MATLAB program which calculates $\Delta F/F$ of the region of interest. $\Delta F/F$ was calculated by three steps. First, rolling average of 4 frames was done to reduce the noise. Next, the average fluorescence (F) was subtracted by the baseline fluorescence ($F_0$) which was the mean of fluorescence from the lowest 6 frames (10%) among the previous 60 frames. Finally, $\Delta F/F \equiv \frac{F-F_0}{F_0}$ was calculated.

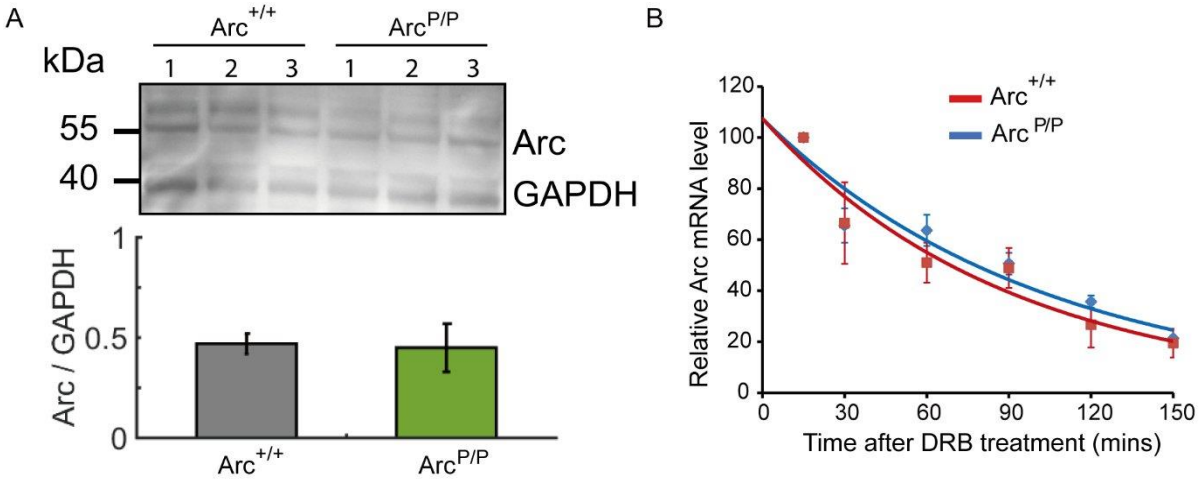


**fig. S1. Comparison of homozygous Arc-PBS mouse to WT mouse. (A)** Western blot image of Arc protein (55 kDa) and GAPDH protein (36 kDa) in brain tissue lysates from three WT ($Arc^{+/+}$) and three homozygous Arc-PBS ($Arc^{P/P}$) knock-in mice (upper panel). Relative Arc expression quantified using GAPDH as loading control was compared between WT and $Arc^{P/P}$ mice (lower panel). No significant difference was observed ($P > 0.05$, n = 3 mice). Error bars represent standard deviation (SD). **(B)** Measurements of Arc mRNA lifetime in bicuculline stimulated hippocampal neurons (75,000 cells/Mattek dish) from WT and $Arc^{P/P}$ mice after DRB treatment. Error bars indicate SEM (n = 3 experiments with 2 replicates).

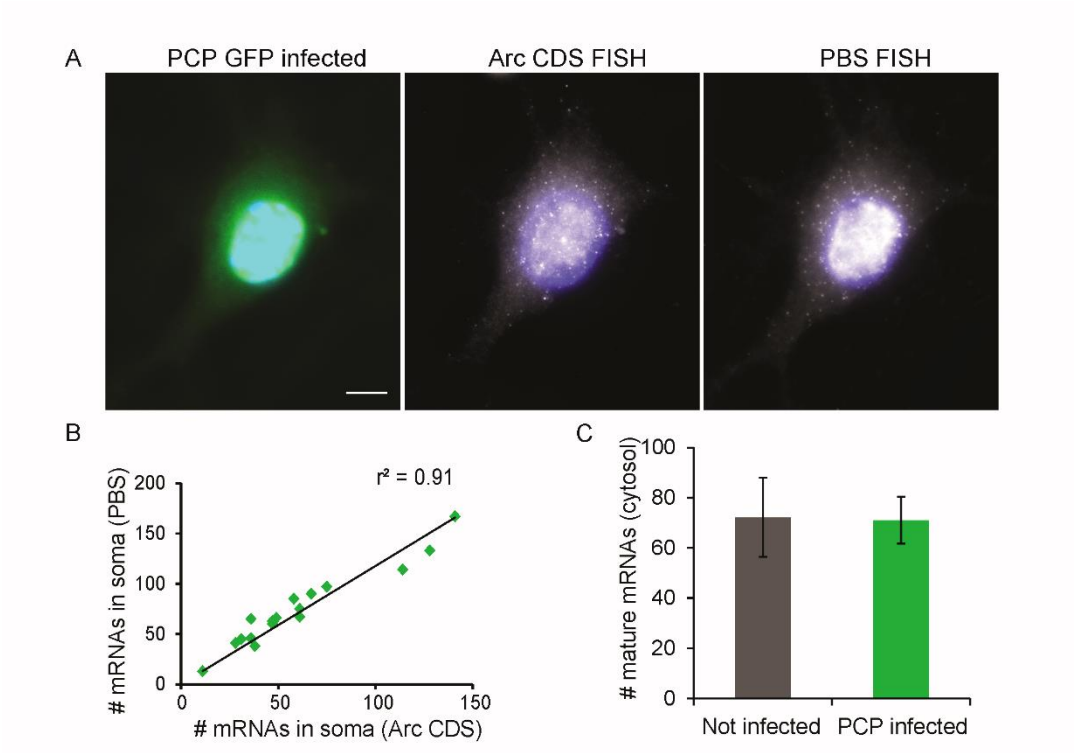


**fig. S2. PCP binding to PBS-tagged mRNAs does not alter mRNA integrity.** Hippocampal neurons from $Arc^{P/P}$ mouse infected with lentivirus expressing tdPCP-tdGFP for 7 days. **(A)** smFISH using Arc CDS and PBS probes in neurons 60 min post bicuculline stimulation. **(B)** Correlation between number of mRNAs detected by Arc CDS and PBS smFISH in PCP-infected neurons, indicated by Pearson's coefficient ($r^2$) (n = 17 neurons). **(C)** Quantification of average number of mRNAs detected in uninfected and PCP-infected neurons from the same culture. Error bars indicate SEM. Scale bar, 5 μm.

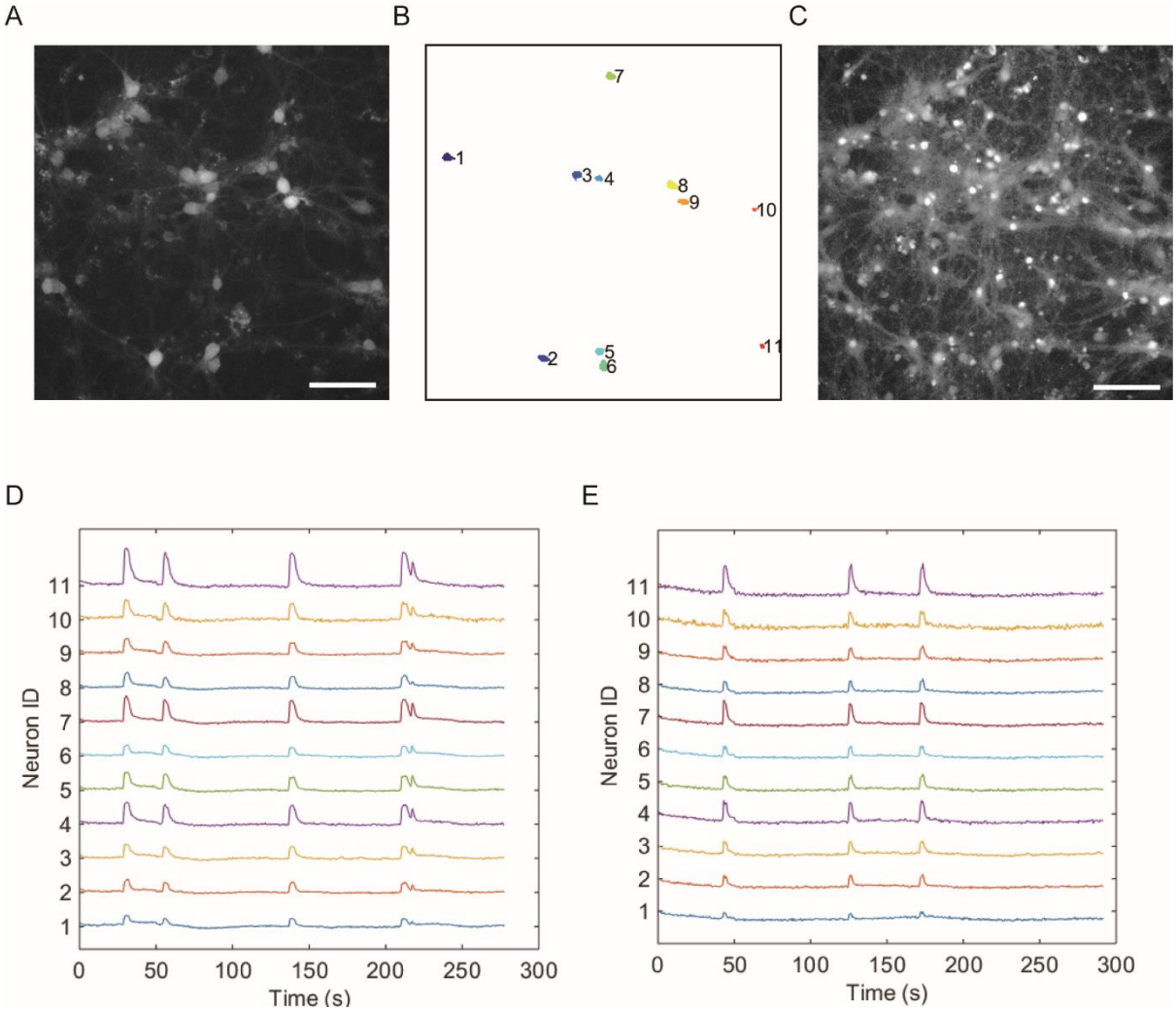


**fig. S3. Persistent synchronized $Ca^{2+}$ spikes after bicuculline washout.** After incubation of neurons with bicuculline (50 μM) for 20 min, bicuculline was washed out. The $Ca^{2+}$ activity after washout of bicuculline was measured. **(A)** Green channel image showing AAV-hSyn-NLS-tdPCP-tdGFP infected neurons (DIV 18) imaged using a 20× magnification objective. **(B)** Selected areas in somas of GFP positive neurons to be analyzed using FluoroSNNAP program. **(C)** Red channel image showing Calcium Orange stained cells. **(D)** Synchronized $Ca^{2+}$ spikes 20 min after washout of bicuculline. **(E)** Synchronized $Ca^{2+}$ spikes 60 min after washout of bicuculline. Scale bars, 50 μm.

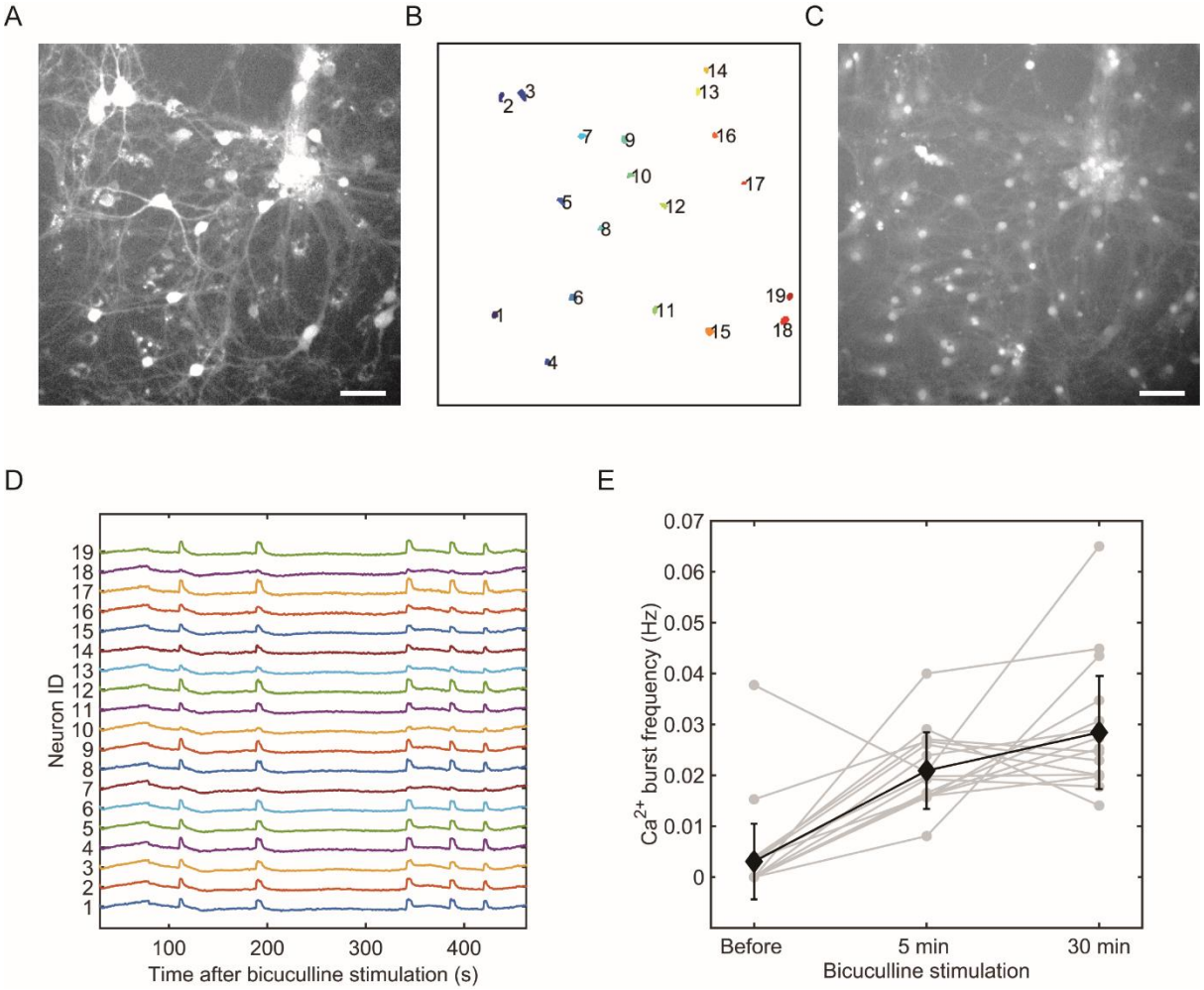


**fig. S4. Synchronized $Ca^{2+}$ spikes during bicuculline incubation. (A)** Green channel image showing AAV-hSyn-NLS-tdPCP-tdGFP infected neurons (DIV 14) imaged using a 20× magnification objective. **(B)** Selected areas in somas of GFP positive neurons to be analyzed using FluoroSNNAP program. **(C)** Red channel image showing Calcium Orange stained cells. **(D)** Synchronized $Ca^{2+}$ spikes induced by 50 μM bicuculline application. Most of neurons exhibit synchronized $Ca^{2+}$ spikes. **(E)** Time course of $Ca^{2+}$ burst frequency change during bicuculline stimulation (n = 46 neurons from 2 different cultures). Error bars represent SD. Scale bars, 50 μm.

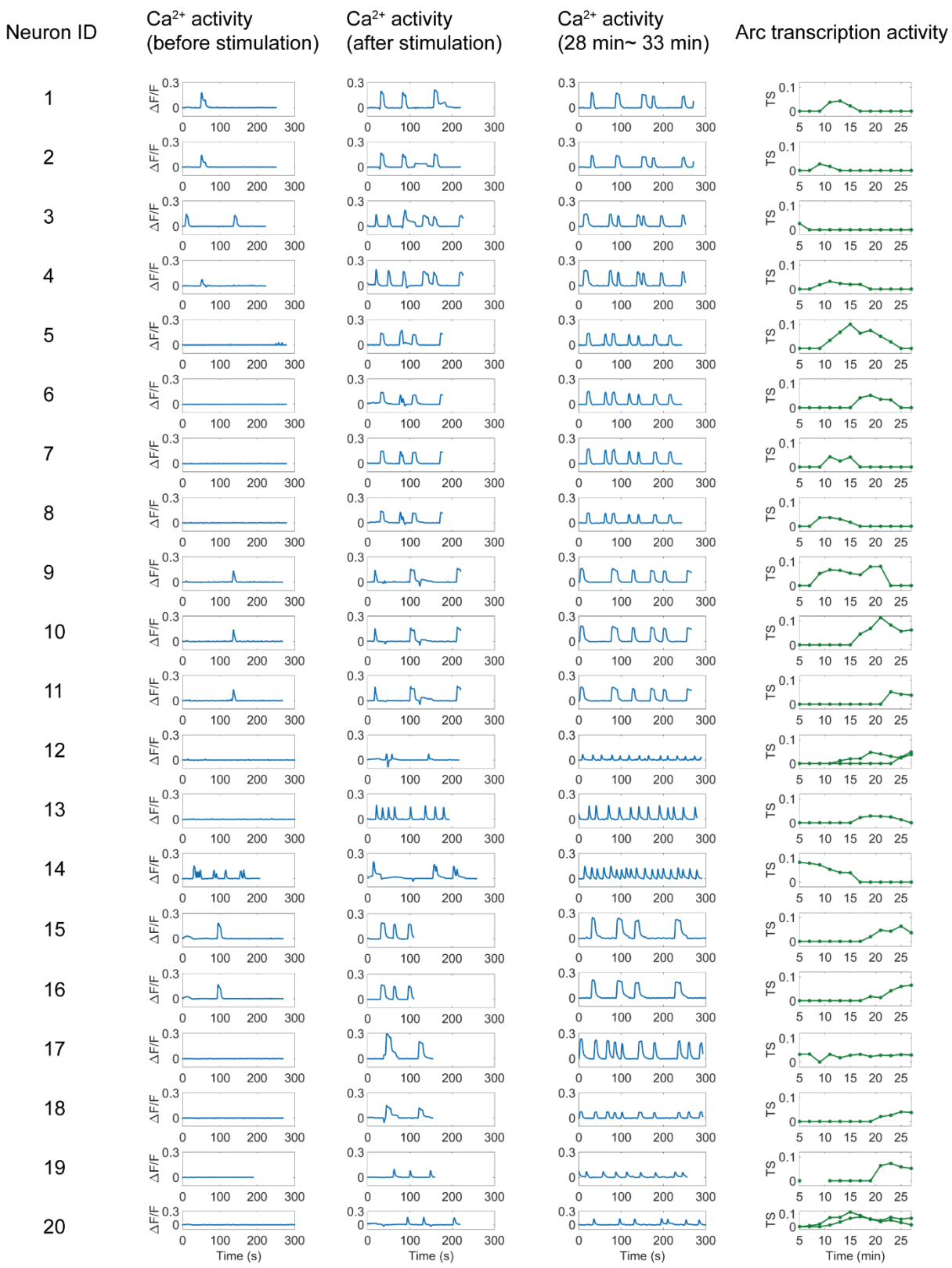


**fig. S5. Plot of $Ca^{2+}$ activity and Arc transcription dynamics.** Data set of transcription-induced neurons. First column corresponds to $Ca^{2+}$ activity before stimulation (basal condition). Second column corresponds to $Ca^{2+}$ activity within 5 min after bicuculline application except for neuron 19. For neuron 19, $Ca^{2+}$ activity of second column was measured during 7-10.5 min after stimulation. Thus, Arc transcription data for neuron 19

was taken from 11 min after stimulation. Third column corresponds to $Ca^{2+}$ activity during 28-33 min after stimulation. The last column shows Arc transcription activity during 5-27 min after stimulation.

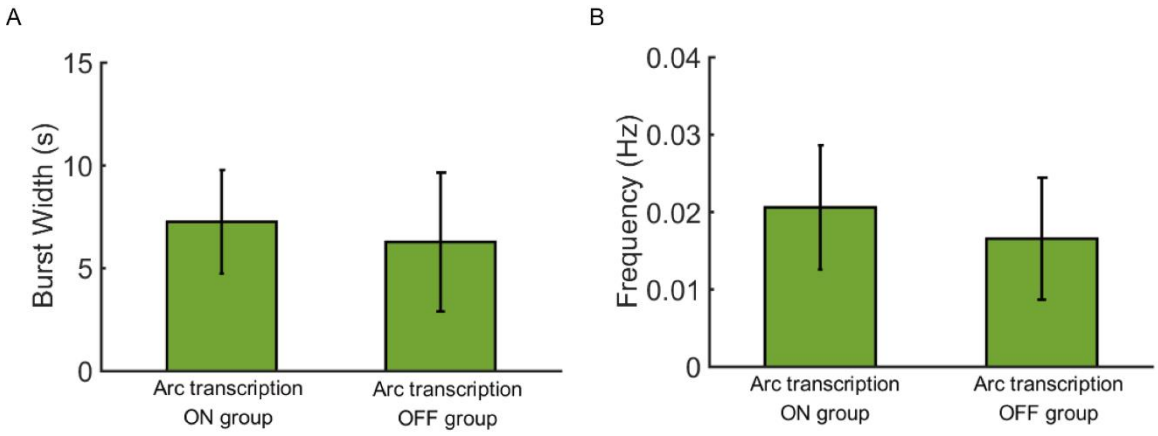


**fig. S6. Comparison of somatic $Ca^{2+}$ spikes between neurons with and without Arc transcription within 30 min after bicuculline stimulation. (A)** Comparison of the average FWHM of $Ca^{2+}$ spikes. No significant difference was observed between the groups with Arc transcription induction (ON) and without Arc transcription (OFF) ($P_{ks} > 0.05$, Kolmogorov –Smirnov test). **(B)** Comparison of average frequency of $Ca^{2+}$ spikes. No significant difference was observed ($P_{ks} > 0.05$, Kolmogorov –Smirnov test). Error bars represent SD (n = 20 neurons for Arc transcription ON group, and n = 26 neurons for Arc transcription OFF group).

**table S1. Sequence of the probes used for smFISH against the Arc-CDS and PBS linkers (PBS).** Probe sets are modified at both 5' and 3'ends (red) for conjugation to dye of choice. CDS probes are labeled with Quasar 570 (Biosearch). The PBS probes were obtained from Biosearch, and labeled in house with Cy3 or Cy5 dyes. Controls for probes have been shown in fig. S2C.

| Probe Name/No. | Sequence (20nt) | Dye |
|---|---|---|
| Arc CDS-1 | **t**taccaatctgcaggatca**c** | Quasar 570 |
| Arc CDS-2 | **t**tggacacttcggtcaaca**g** | Quasar 570 |
| Arc CDS-3 | **c**aagttgttctccagcttg**c** | Quasar 570 |
| Arc CDS-4 | **c**aaagacaggccttgatgg**a** | Quasar 570 |
| Arc CDS-5 | **a**cgtgcatctcacgcttga**c** | Quasar 570 |
| Arc CDS-6 | **c**tctccagacggtagaaga**c** | Quasar 570 |
| Arc CDS-7 | **a**cacctacagagacagtgt**g** | Quasar 570 |
| Arc CDS-8 | **t**gatggcataggggctaac**g** | Quasar 570 |
| Arc CDS-9 | **c**ccaagactgatattgctg**a** | Quasar 570 |
| Arc CDS-10 | **c**tcgaagatctgtgtatcc**a** | Quasar 570 |
| Arc CDS-11 | **c**agccaatattcttcagag**c** | Quasar 570 |
| Arc CDS-12 | **t**gaactcccaccacttctt**g** | Quasar 570 |
| Arc CDS-13 | **a**aactccttcttgaactcc**a** | Quasar 570 |
| Arc CDS-14 | **t**gaatggcttcacgggaga**g** | Quasar 570 |
| Arc CDS-15 | **c**agaggaactggtcgagtg**g** | Quasar 570 |
| Arc CDS-16 | **c**atacagtgtctggtacag**g** | Quasar 570 |
| Arc CDS-17 | **c**atactgaatgatctcctc**c** | Quasar 570 |
| Arc CDS-18 | **a**gaaagcgcttgagtttgg**g** | Quasar 570 |
| Arc CDS-19 | **c**tctggataagctgctcca**g** | Quasar 570 |
| Arc CDS-20 | **c**tactgactcgctggtaag**a** | Quasar 570 |
| PBS-1 | **t**ttctagagtcgacctgca**g** | Cy3/Cy5 |
| PBS-2 | **c**taggcaattaggtacctta**g** | Cy3/Cy5 |
| PBS-3 | **c**taatgaacccgggaatact**g** | Cy3/Cy5 |

## Supplementary Movie Captions

**movie S1. Immediate early activation of transcription from PBS-tagged Arc allele.** Time-lapse images of Arc transcription in TTX-addition conditions, and after withdrawal in the same neurons. Images acquired every 90 s. Movie is played at 10 fps.

**movie S2. $Ca^{2+}$ activity of neurons induced by bicuculline stimulation.** Somatic $Ca^{2+}$ activity of neurons stimulated by bicuculline shown in Fig. 5, B and C. Time-lapse images were acquired at 2 fps. Movie is played at 100 fps, which is 50 times faster than real time.

**movie S3. Arc transcription dynamics induced by bicuculline stimulation.** Arc transcription activity of neurons after bicuculline stimulation shown in Fig. 5, D and E. Z-stack images of nucleus were taken every 2 min. The z plane best focused to transcription sites was chosen for movie construction. Movie is played at 6 fps.

**movie S4. Dendritic transport of Arc mRNA.** Arc mRNA movement in the dendrite shown in Fig. 6C. Time-lapse images were acquired at 5 fps. Movie is played at 50 fps, which is 10 times faster than real time.