C5aR-dependent microglia activity

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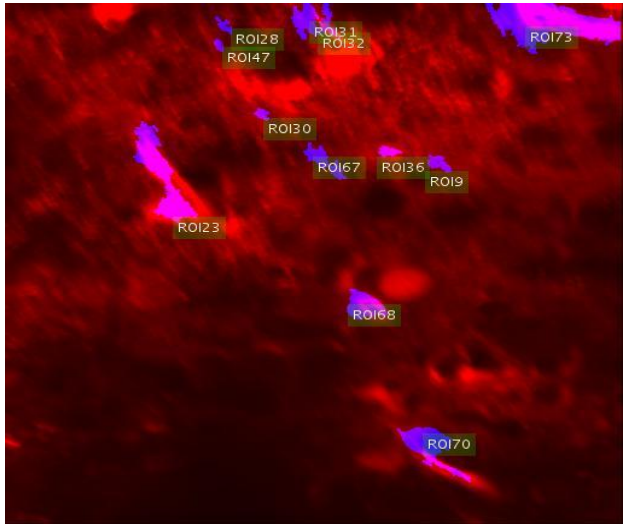
Department seminar
17.02.2023
Kyiv – Iowa City

What is a level of intrinsic spontaneous microglia activity in particular induced by the spinal cord extraction from mice?

Whether C5a application can produce a significant and substantial increase in microglia activity in order to warrant our electrophysiological experiments?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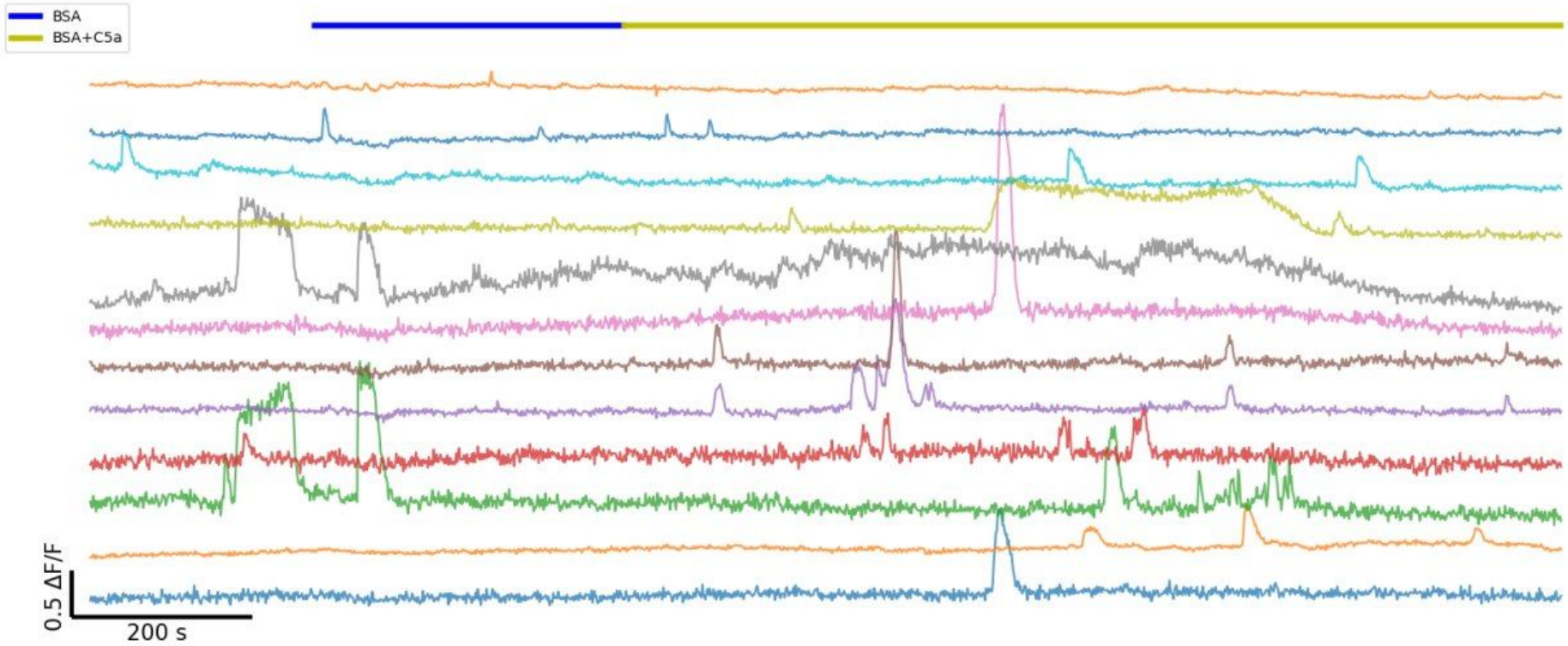
BSA
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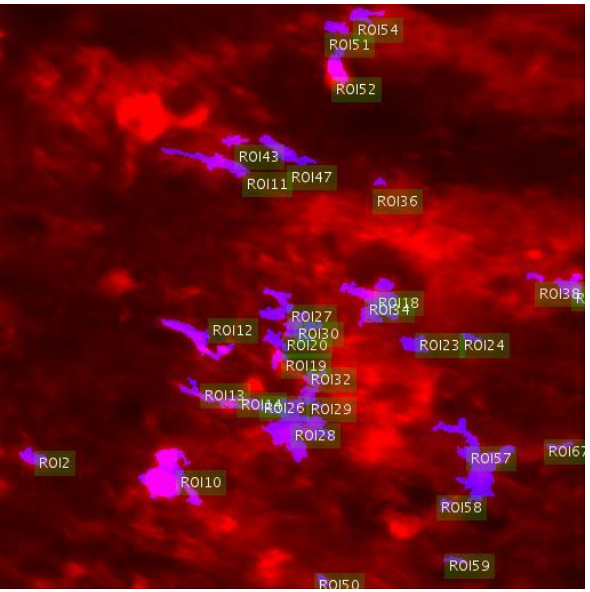
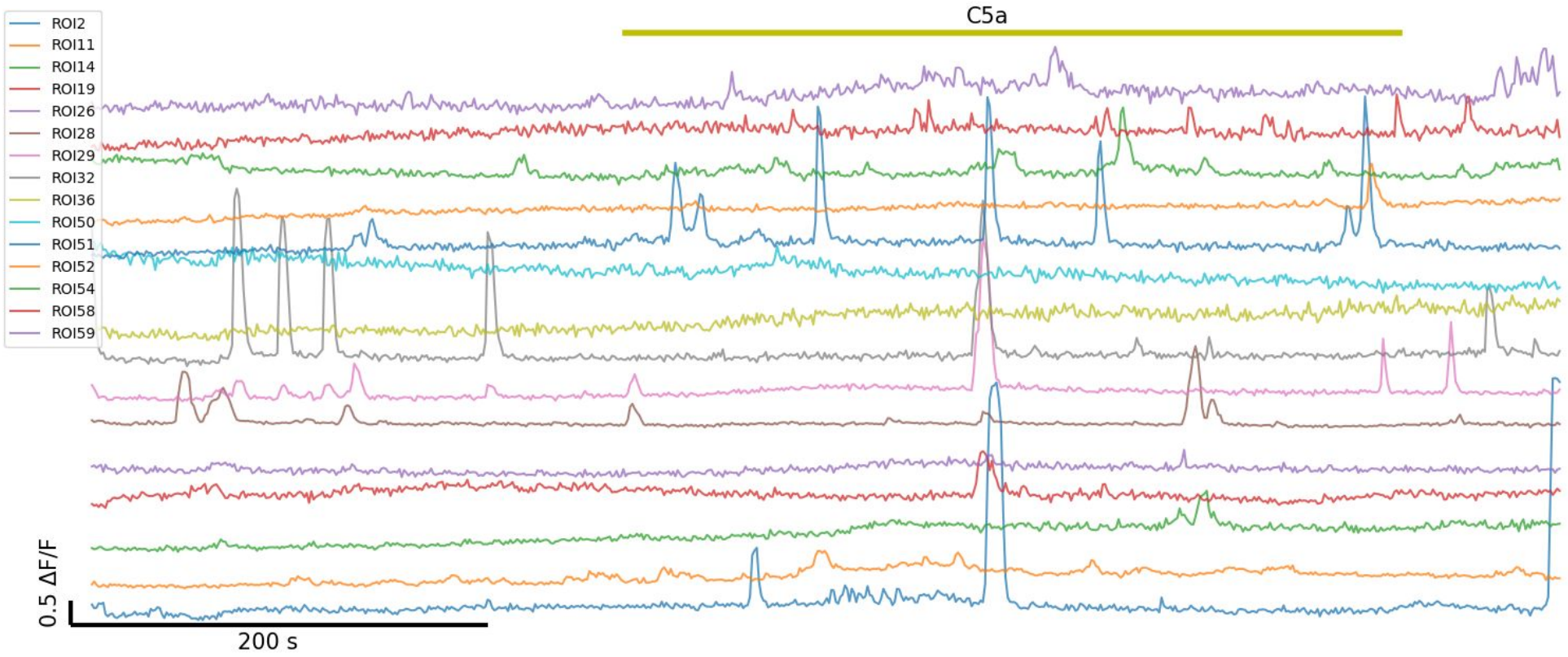
Raw traces E0002, before wash





What is a level of intrinsic spontaneous microglia activity in particular induced by the spinal cord extraction from mice?

Substantial spontaneous microglia activity does exist.

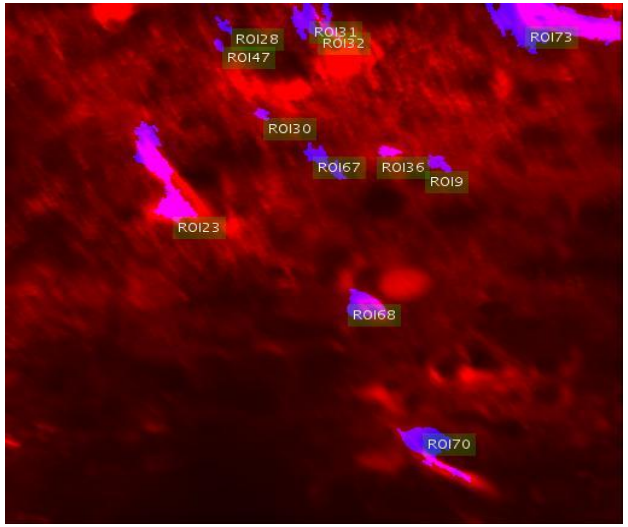
Whether C5a application can produce a significant and substantial increase in microglia activity in order to warrant our electrophysiological experiments?

It is likely that C5aR activation increases the frequency of microglial [Ca2+]i transients 2-3 times. We need to process the results of already performed experiments. Possibly additional experiments will be necessary.

Whether the level of intrinsic spontaneous microglia activity is increased due to the spinal cord extraction from mice over the time course of our electrophysiological experiments (30-60 min)?

We need to conduct 3-5 experiments with 30-60 min recording of spontaneous microglia activity.

Whether perfusion via different syringes and BSA can produce significant changes in microglia activity?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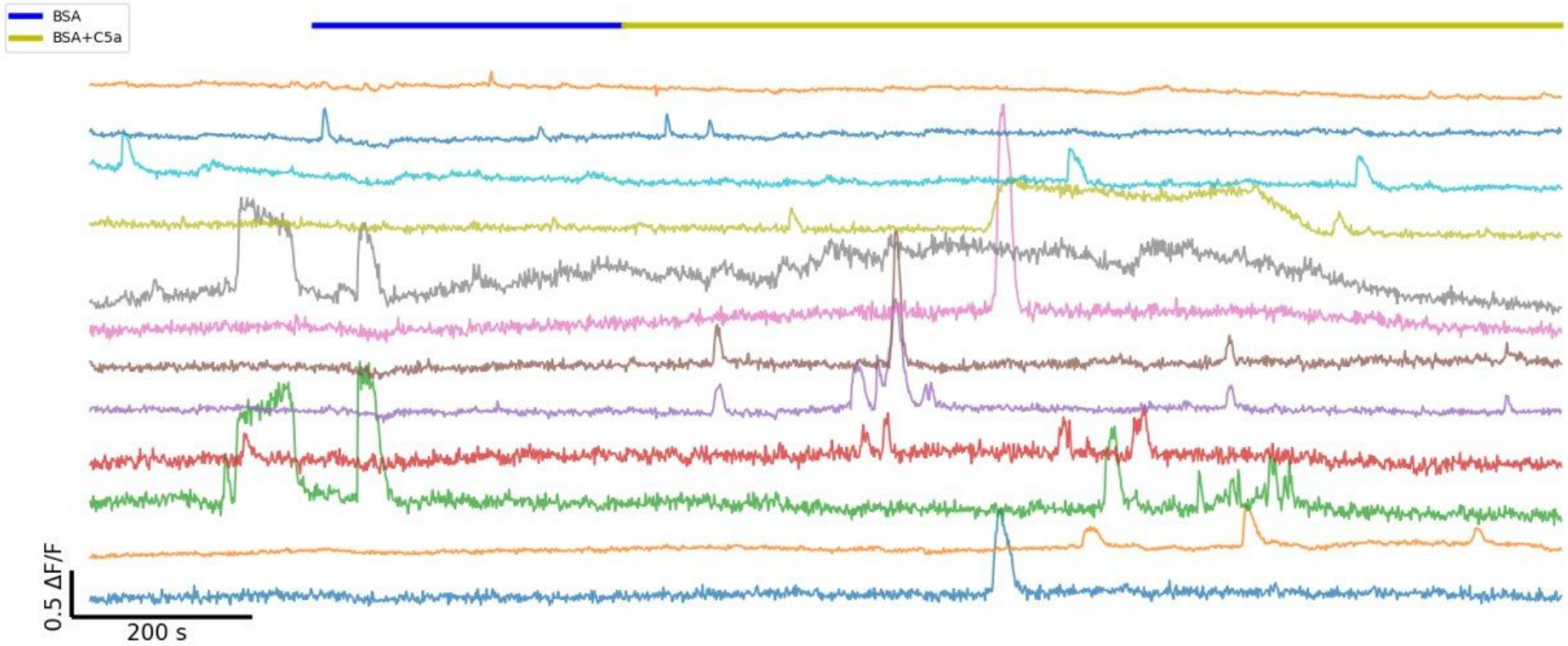
BSA
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0.5 ΔF/F
200 s

Whether perfusion via different syringes and BSA can produce significant changes in microglia activity?

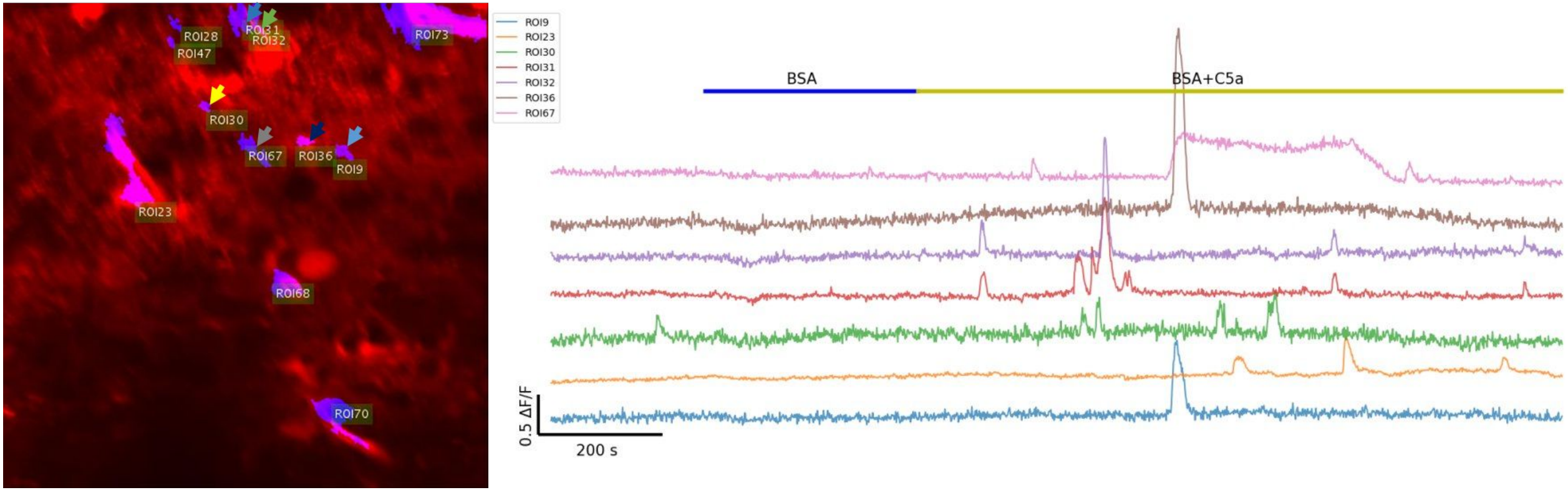
It seems that changes in perfusion lines can create substantial artefacts in a baseline and XY movement. We would recommend to add drugs to the same perfusion line if possible.

How spatially C5a-dependent microglia activity is organized?

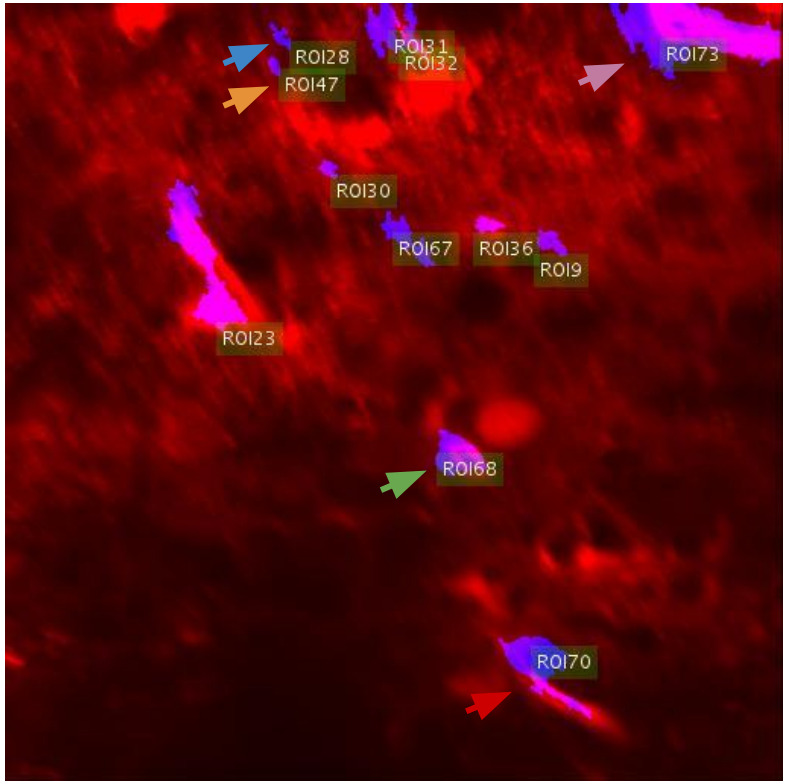
Whether C5a-dependent microglia activity is mainly newly emerged or is it rather increase in frequency of previously spontaneous active sites?

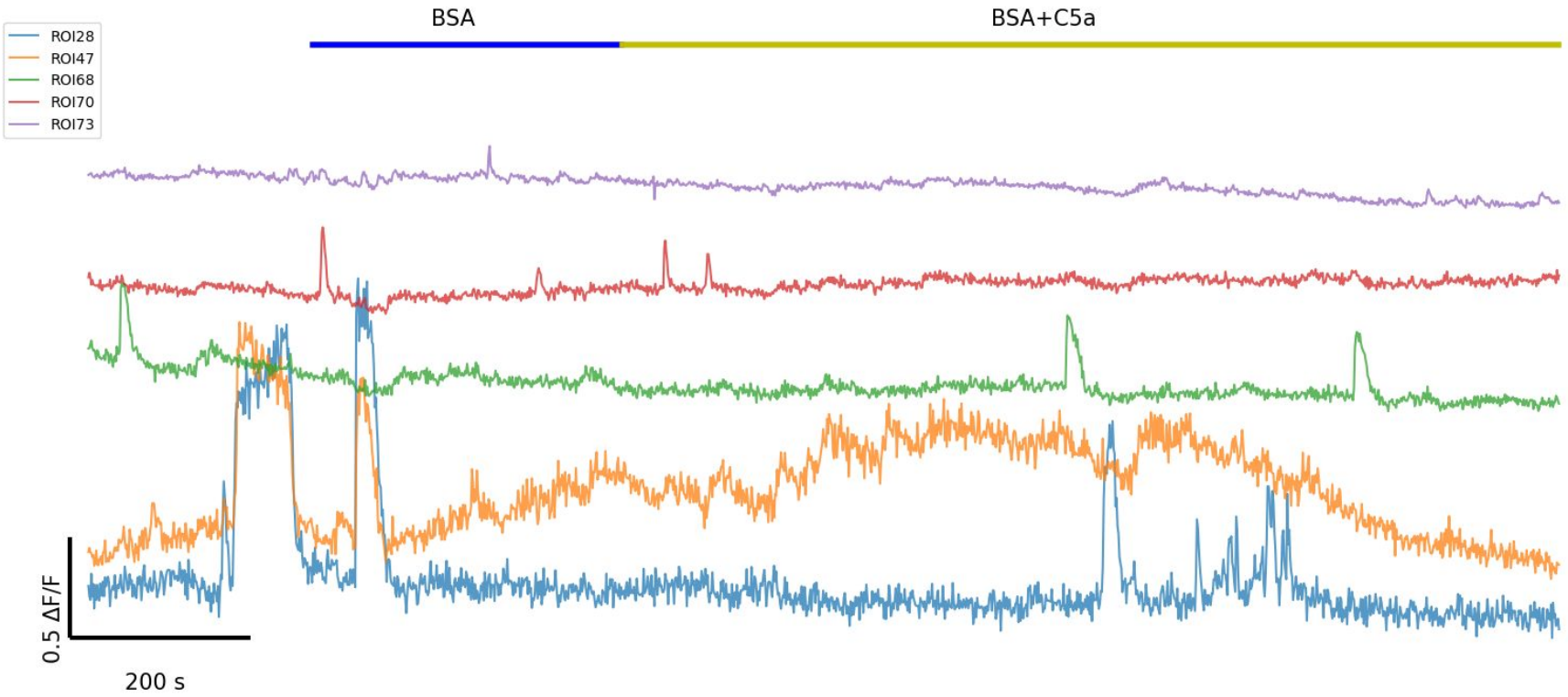
Is C5a-dependent microglia activity randomly distributed over FOV of rather concentrated in certain sites?

Norm traces F7, silent



Norm traces F7, active





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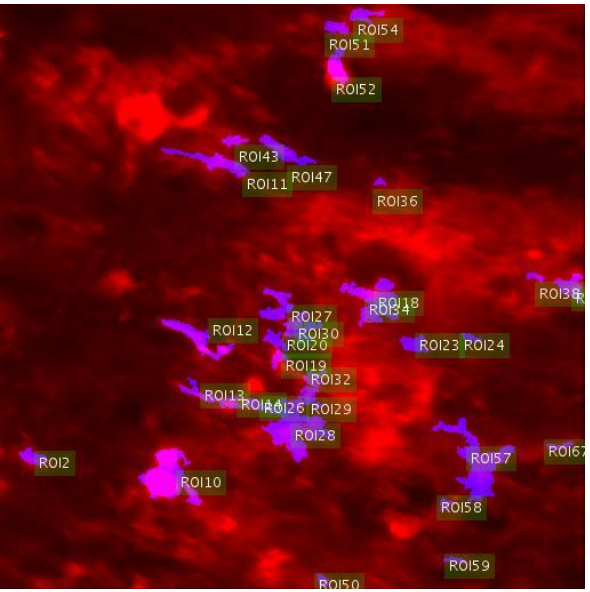

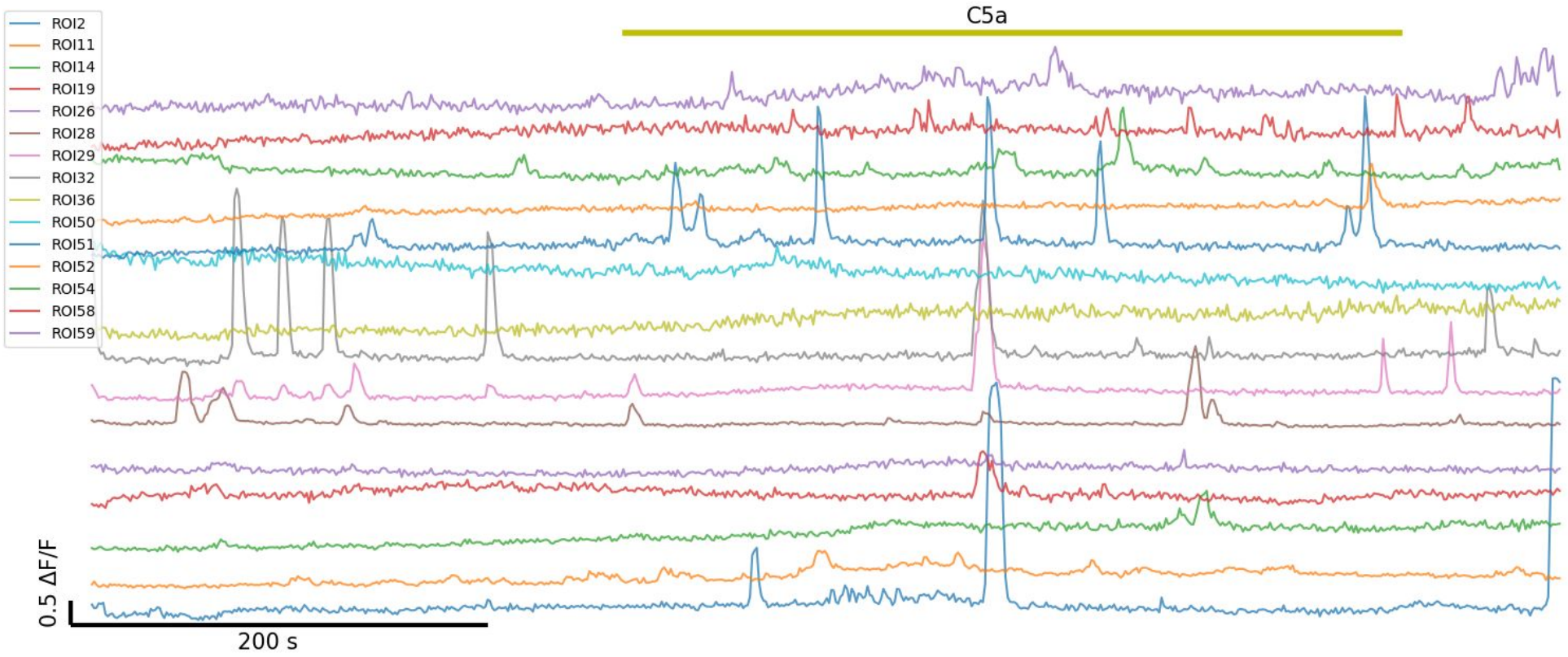

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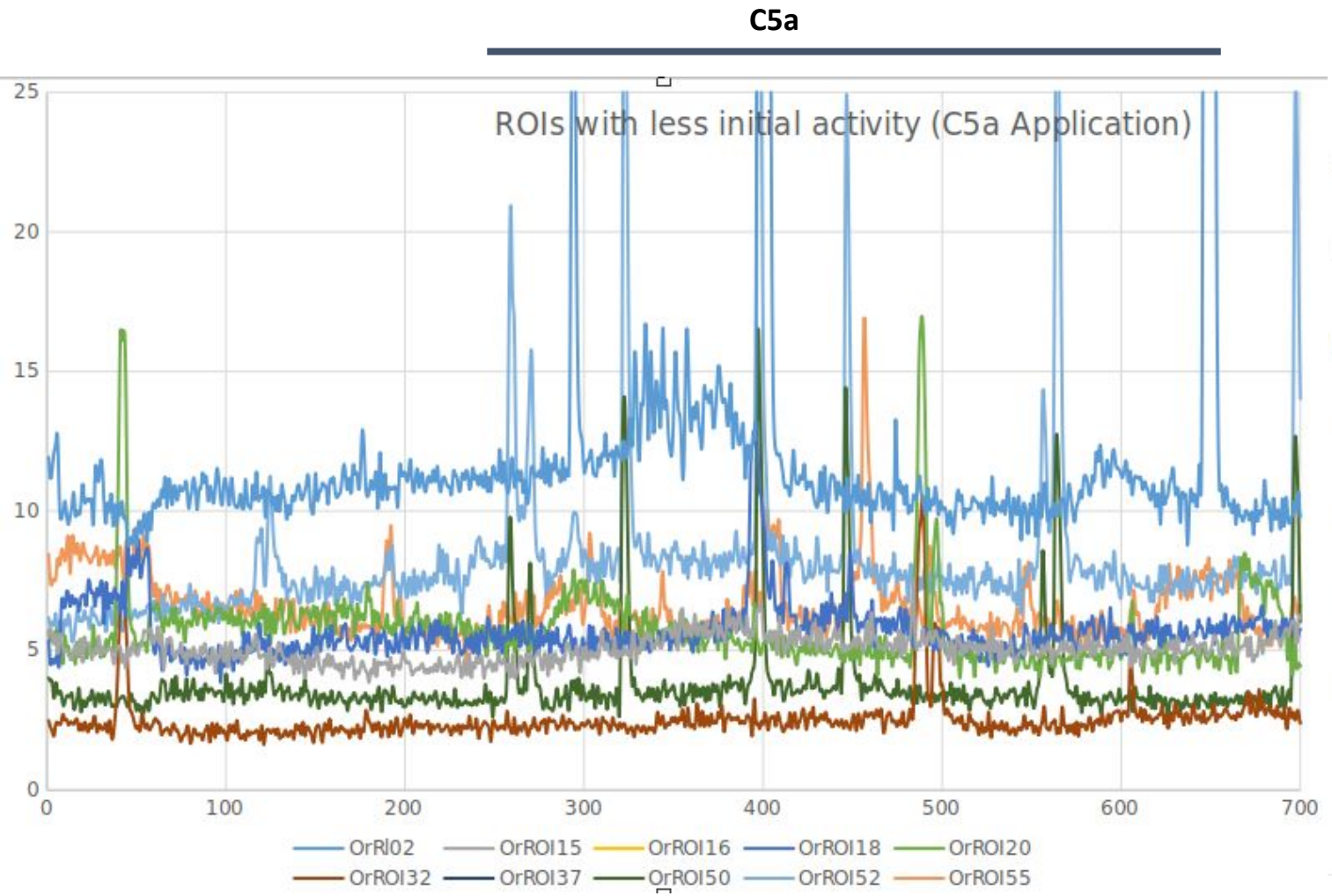
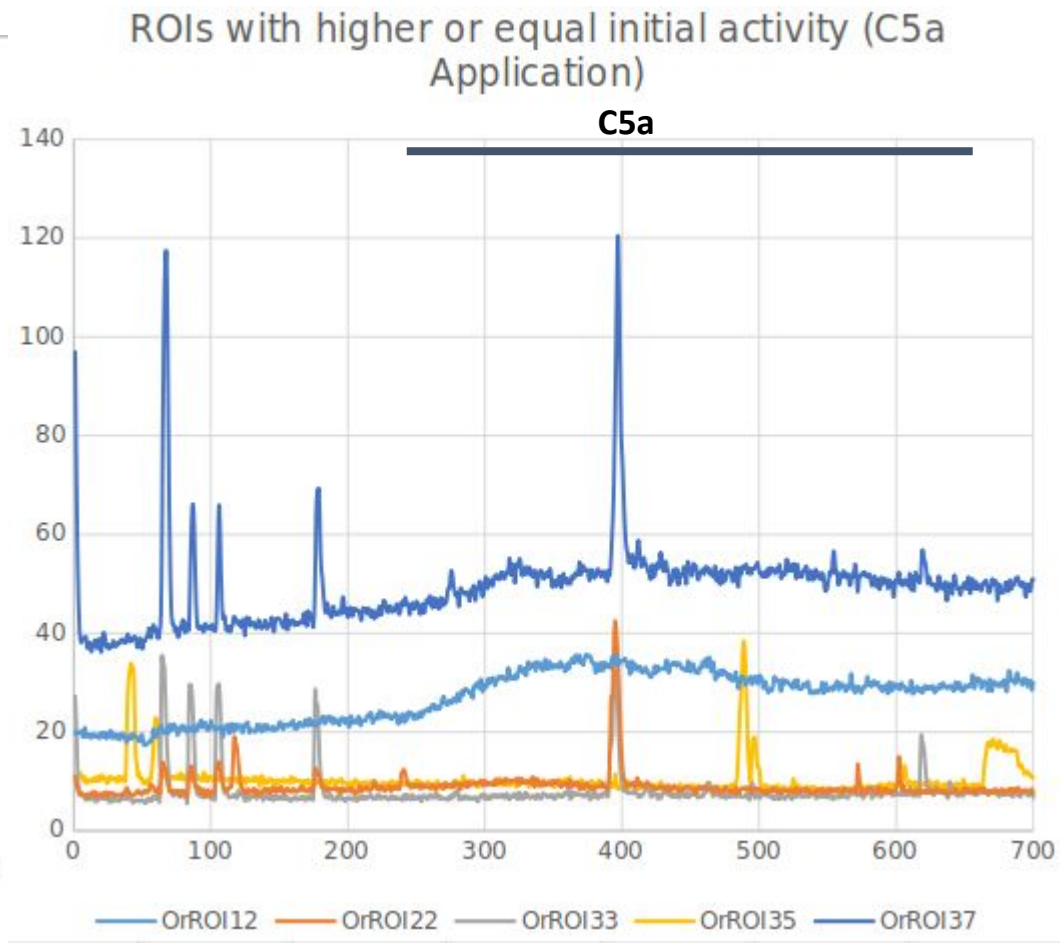




How spatially C5a-dependent microglia activity is organized?

Whether C5a-dependent microglia activity is mainly newly emerged or is it rather increase in frequency of previously spontaneous active sites?

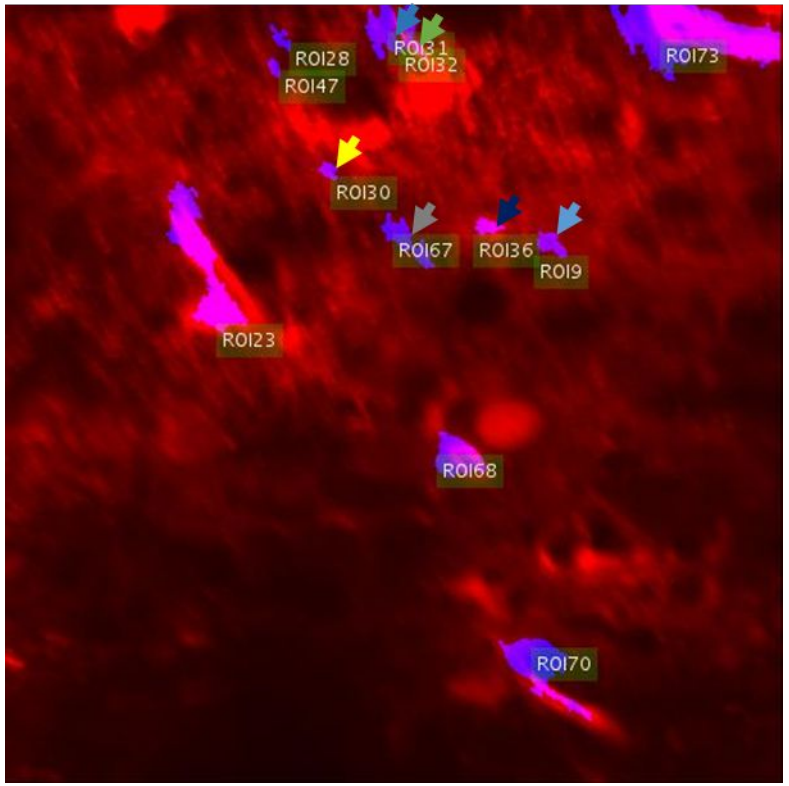
It is likely that the most active sites are newly emerged. Probably there is also increase of activity in spontaneously active sites. Additional experiments should be performed or processed.

Is C5a-dependent microglia activity randomly distributed over FOV of rather concentrated in certain sites?

Possibly regions of microglia activity are segrated. Additional analysis is warranted.

How spatially C5a-dependent microglia activity is organized?

Whether C5a-dependent microglia activity is mainly emerged in soma or processes of microglia cells?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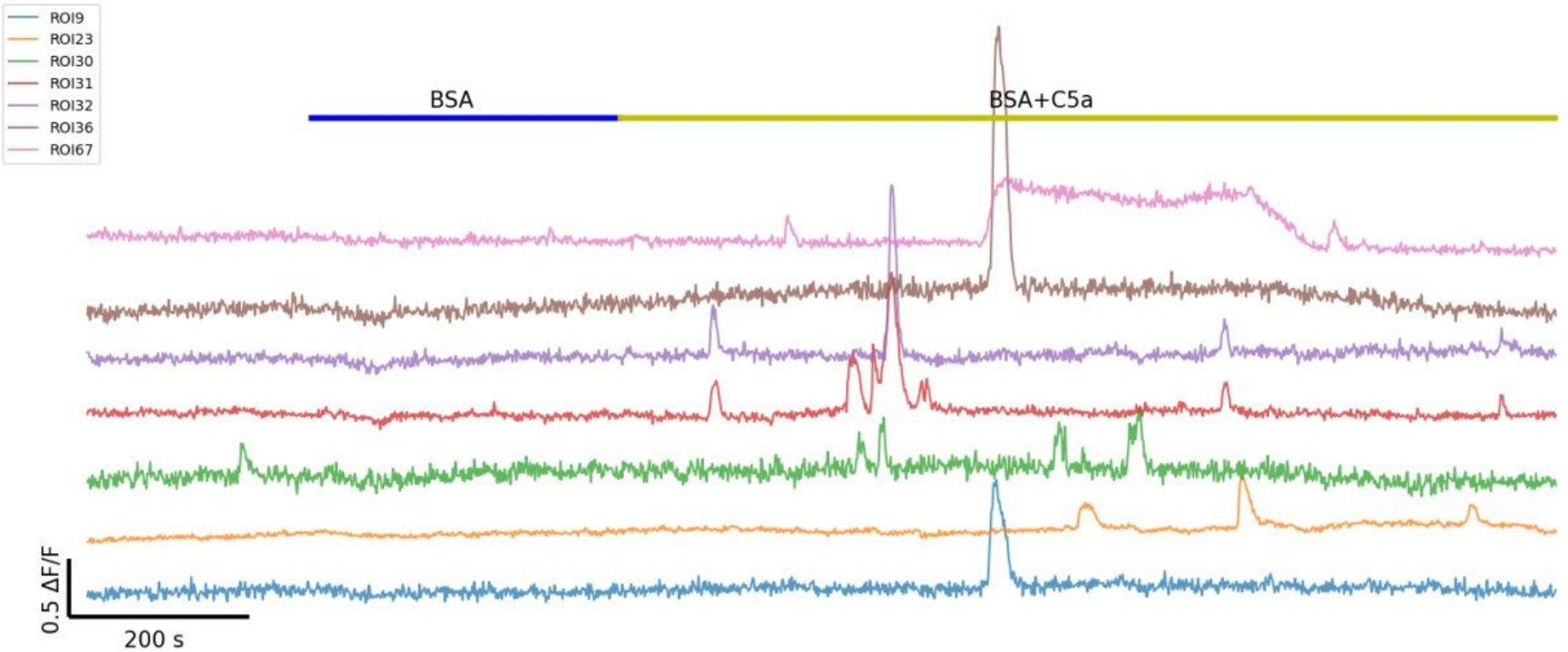
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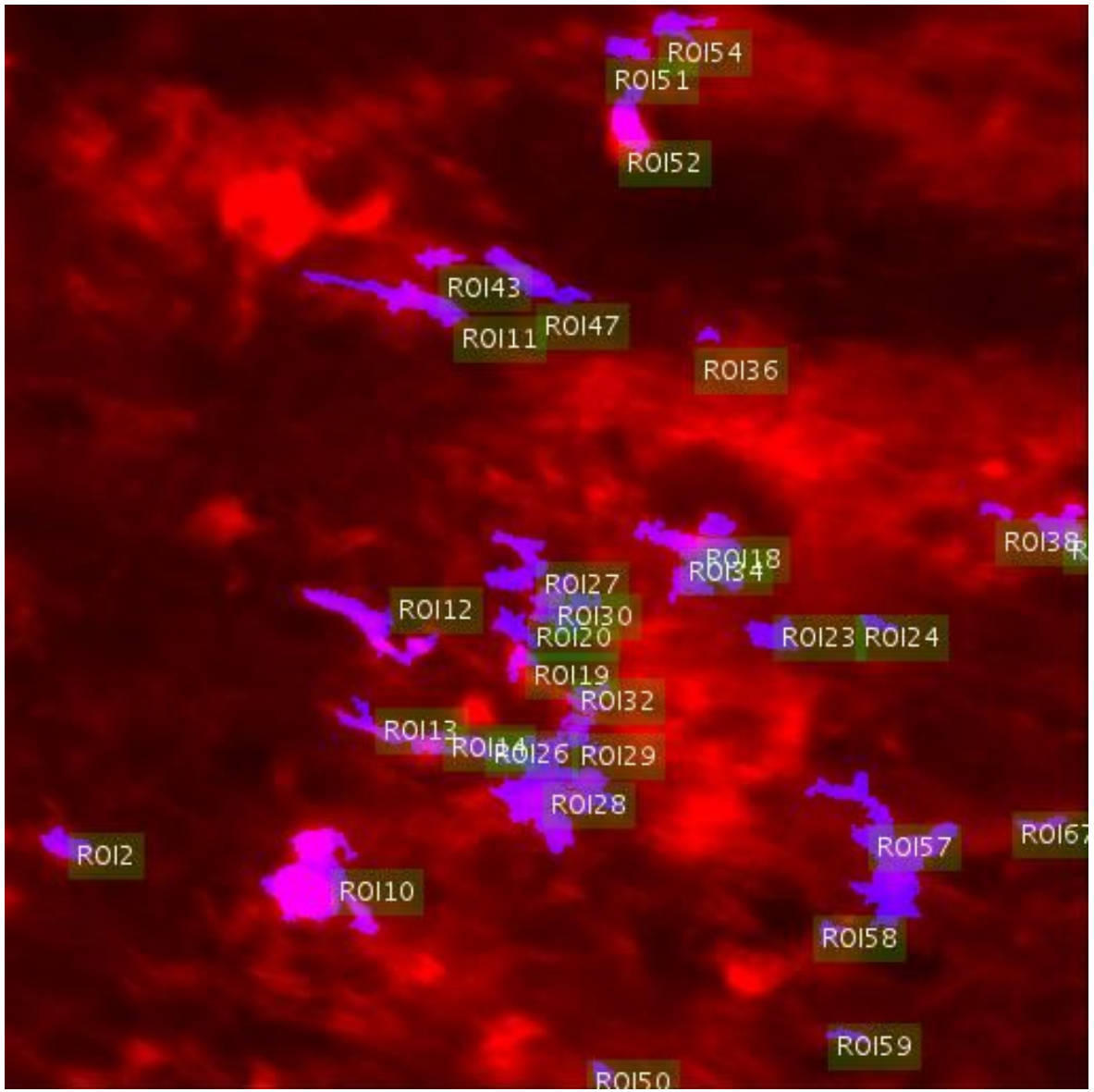
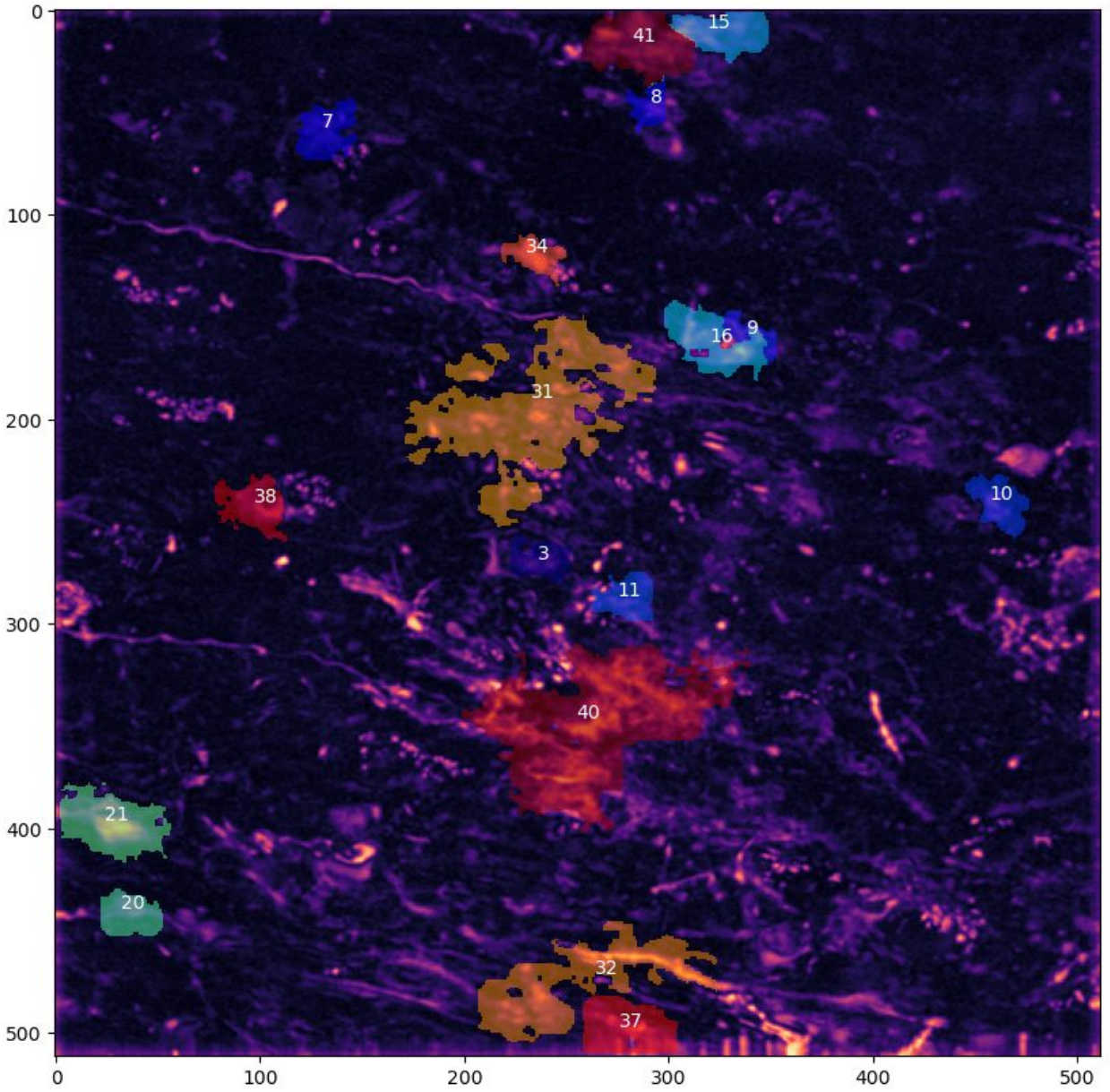
200 s

Separate ROIs



Combined ROIs



How spatially C5a-dependent microglia activity is organized?

Possibly C5a activates microglia in the restricted areas of spinal cord. Thus, neurons located in this area are likely to be affected while other neurons will not change there activity in C5aR-dependent manner.

Whether C5a-dependent microglia activity is mainly emerged in soma or processes of microglia cells?

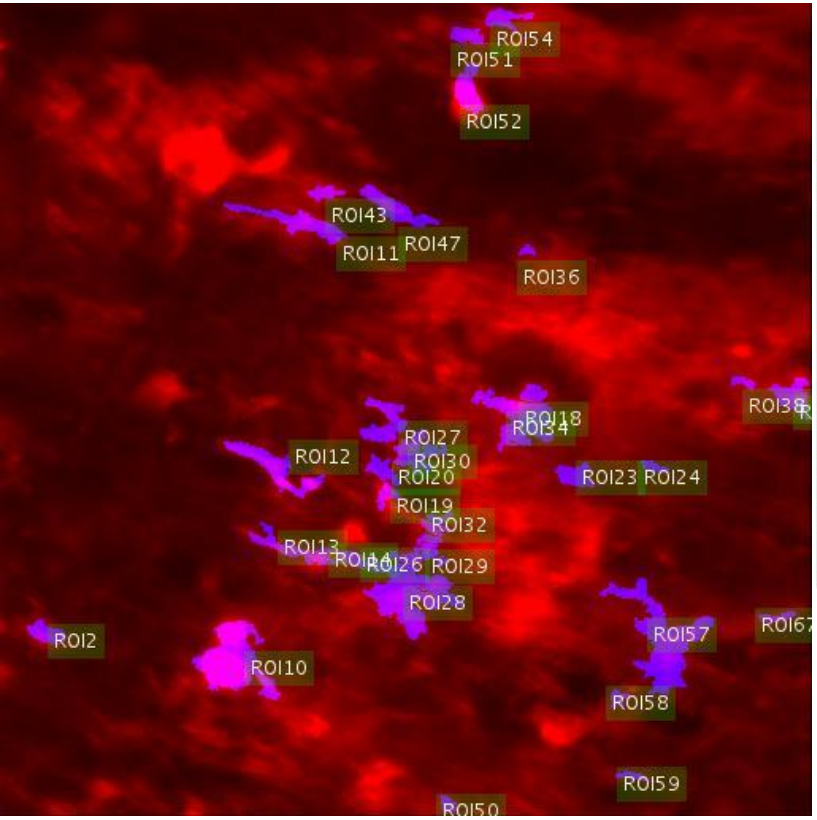
It seems that the effect of C5aR activation is mainly manifested in microglia processes although somas can be also involved. We think we need to show separate statistics for somas and processes.

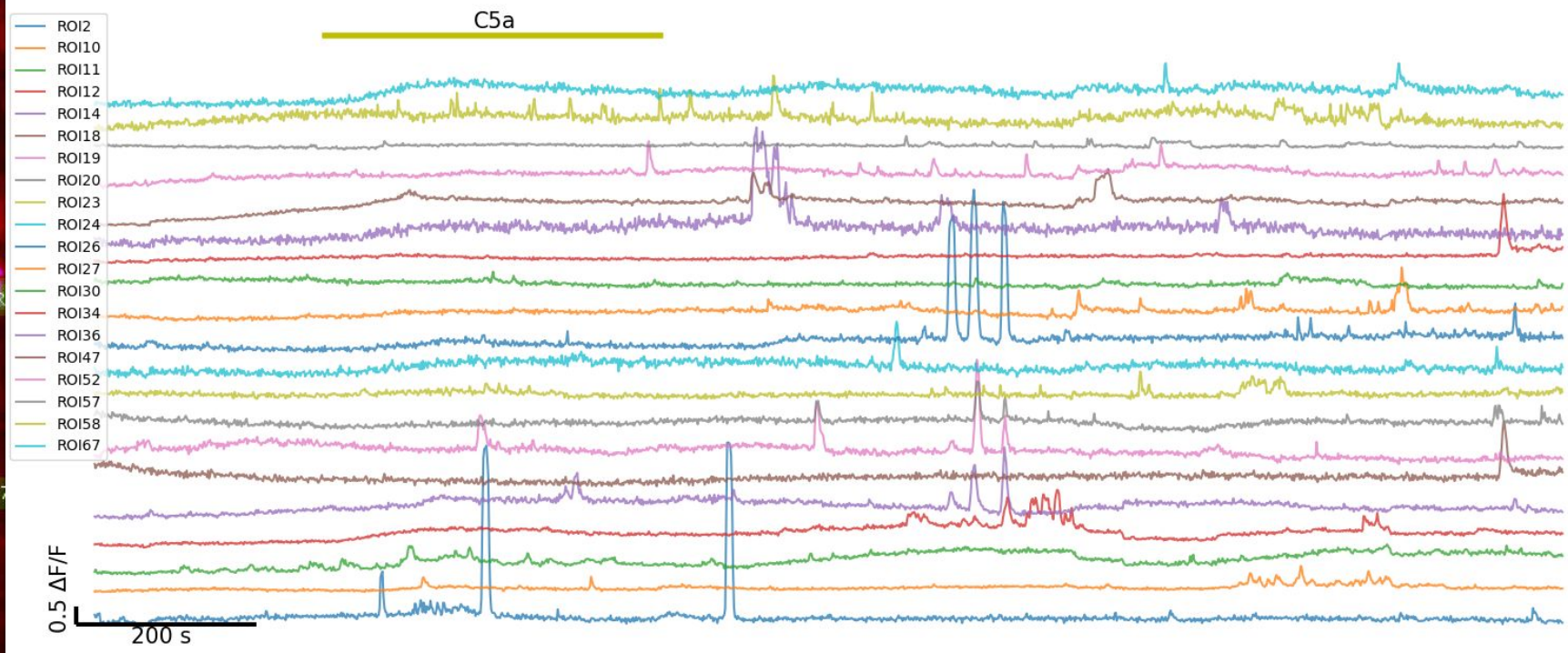
What is a time course of C5a-induced microglia activity?

Is there some desensitization of C5aRs?

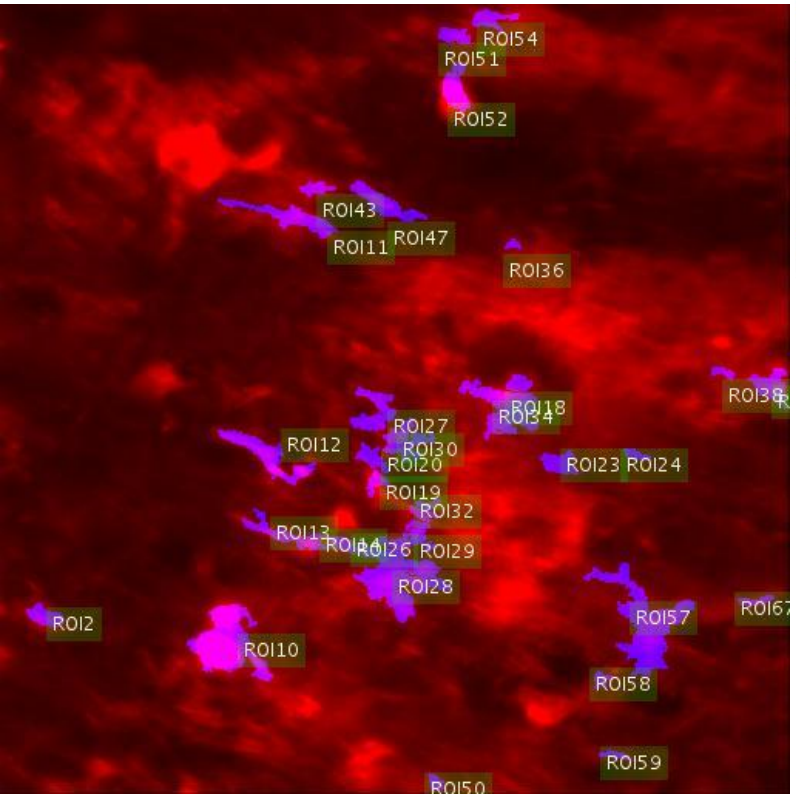
Is the effect of C5a washable?

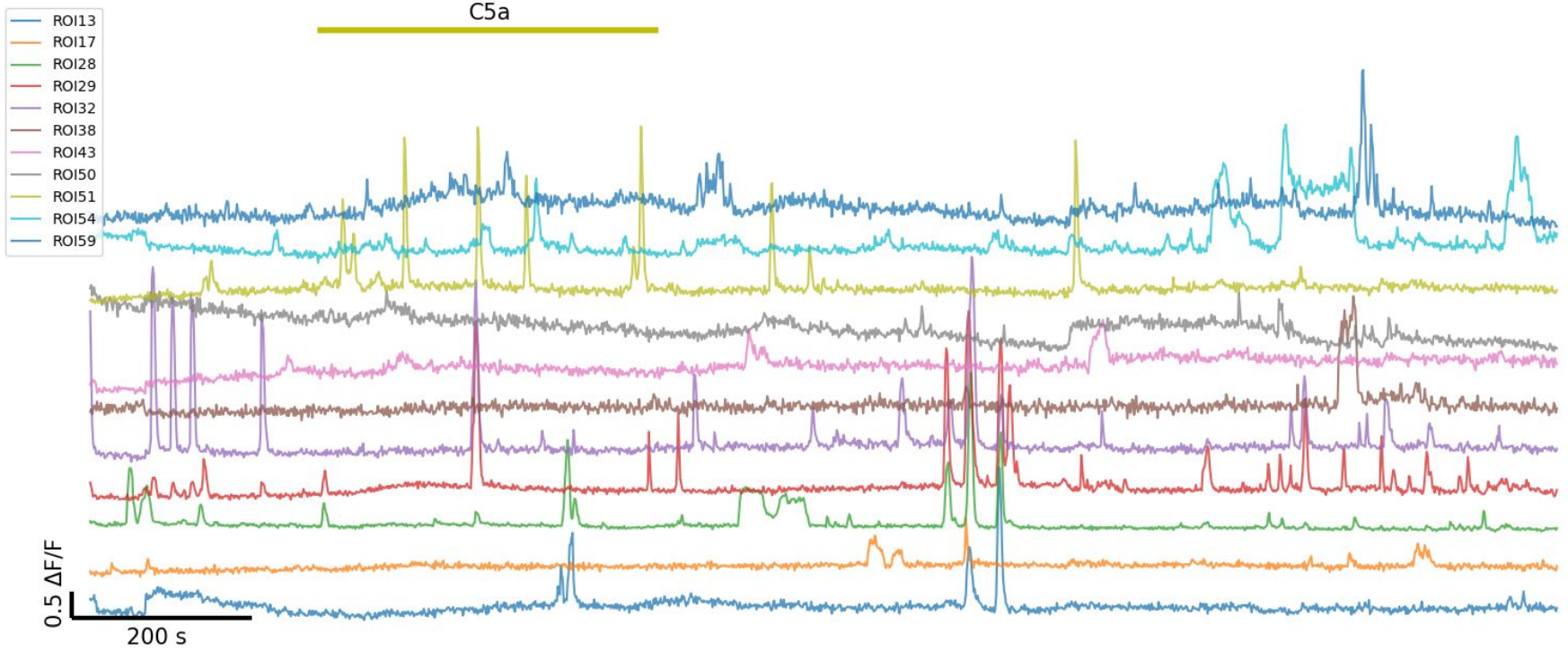
Norm traces E0002, silent



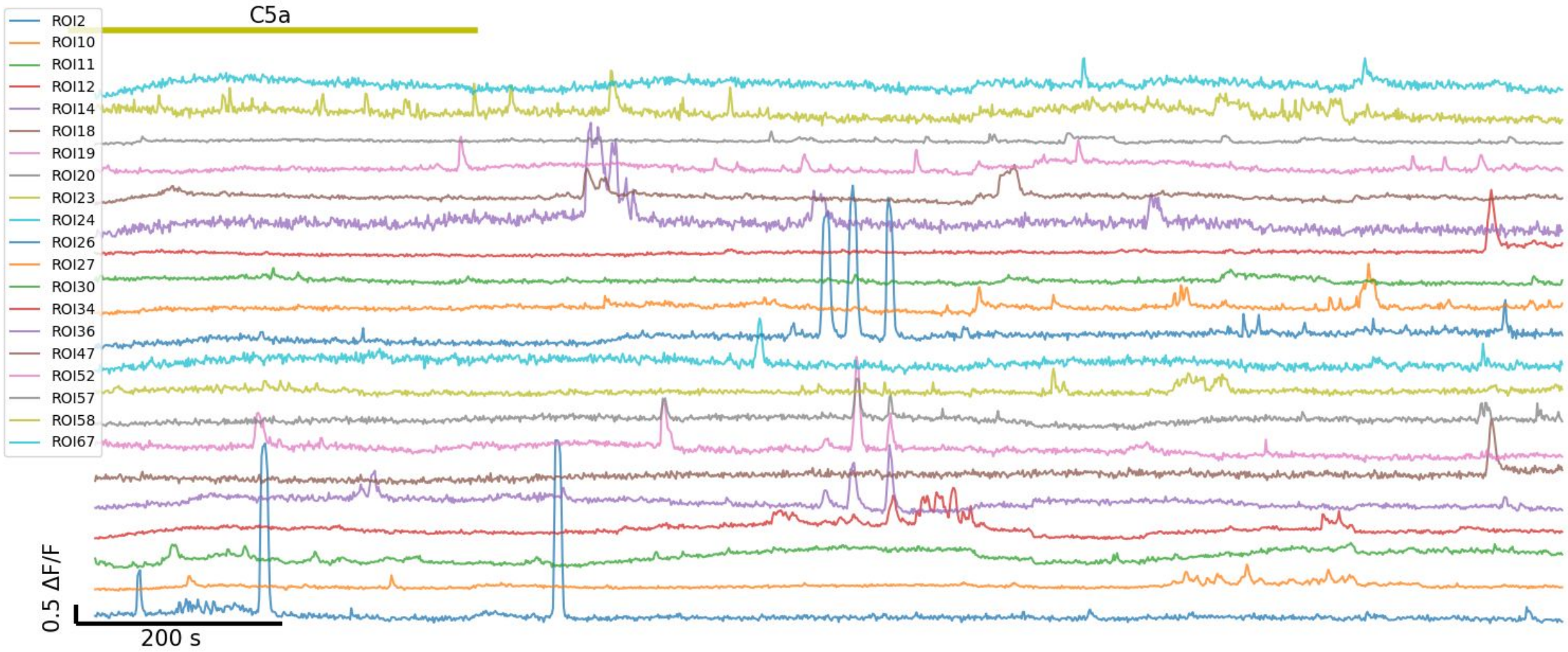


Norm traces E0002, active

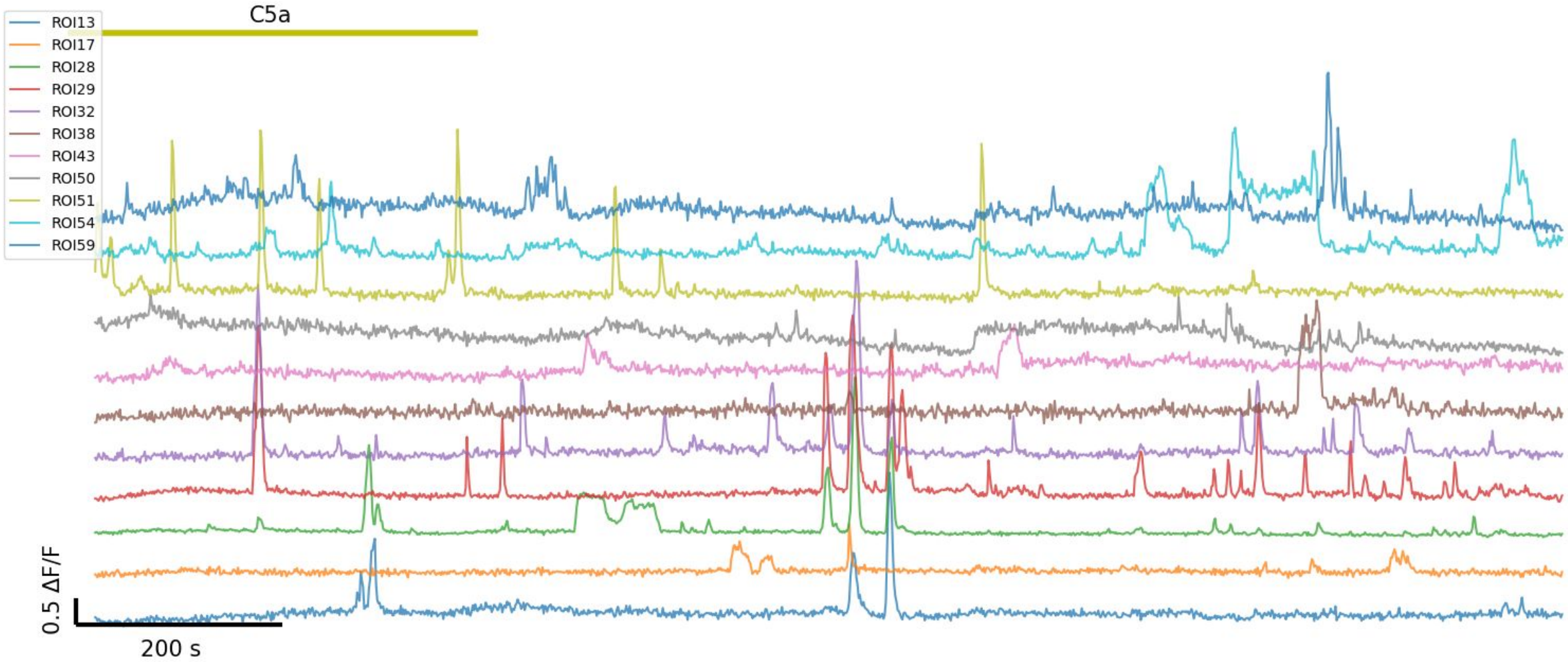




E0002 silent C5a+wash



E0002 before C5a+wash



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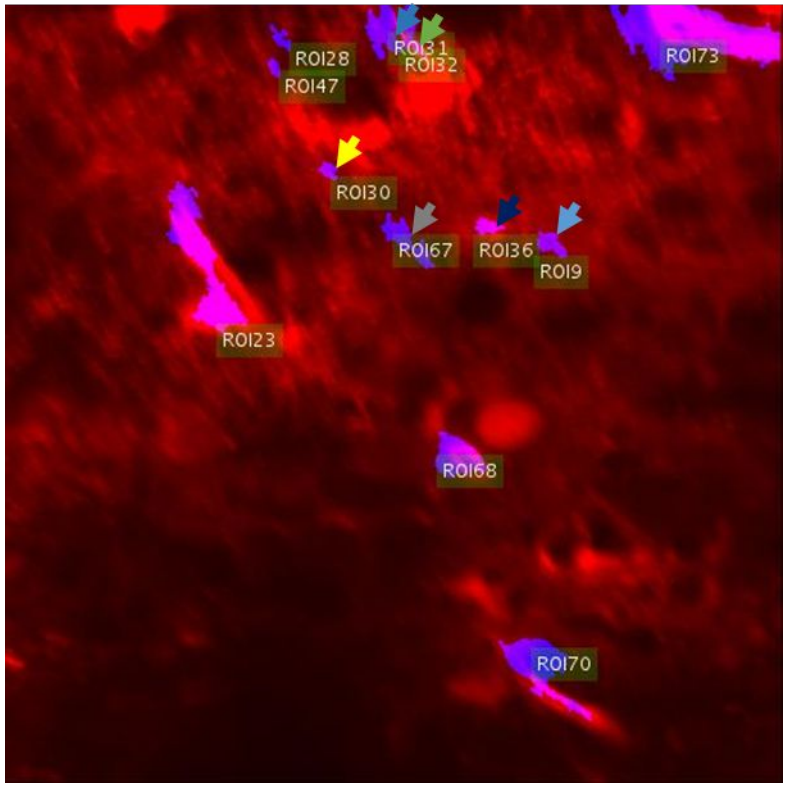
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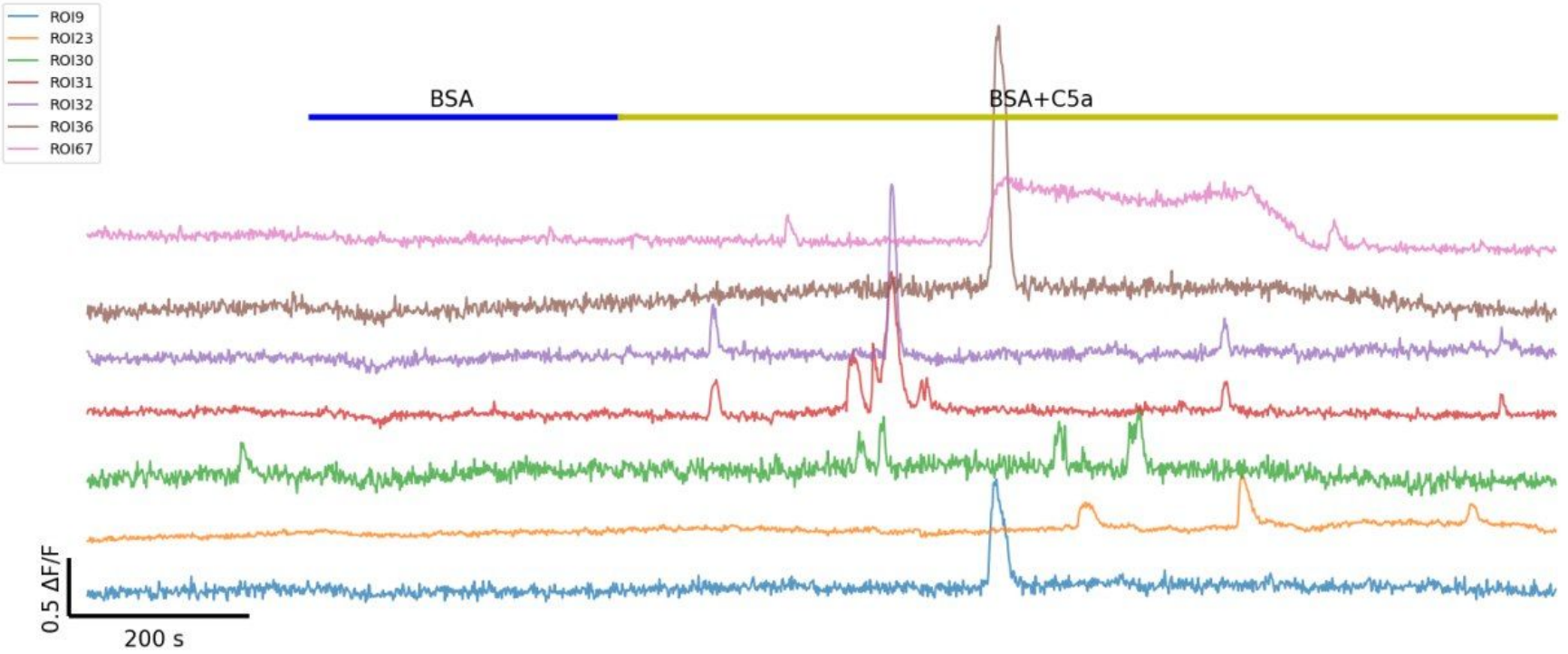
ROI9
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ROI36
ROI67
BSA
BSA+C5a
0.5 ΔF/F
200 s

What is a time course of C5a-induced microglia activity?

The effect is not instantaneous (about 1 min).

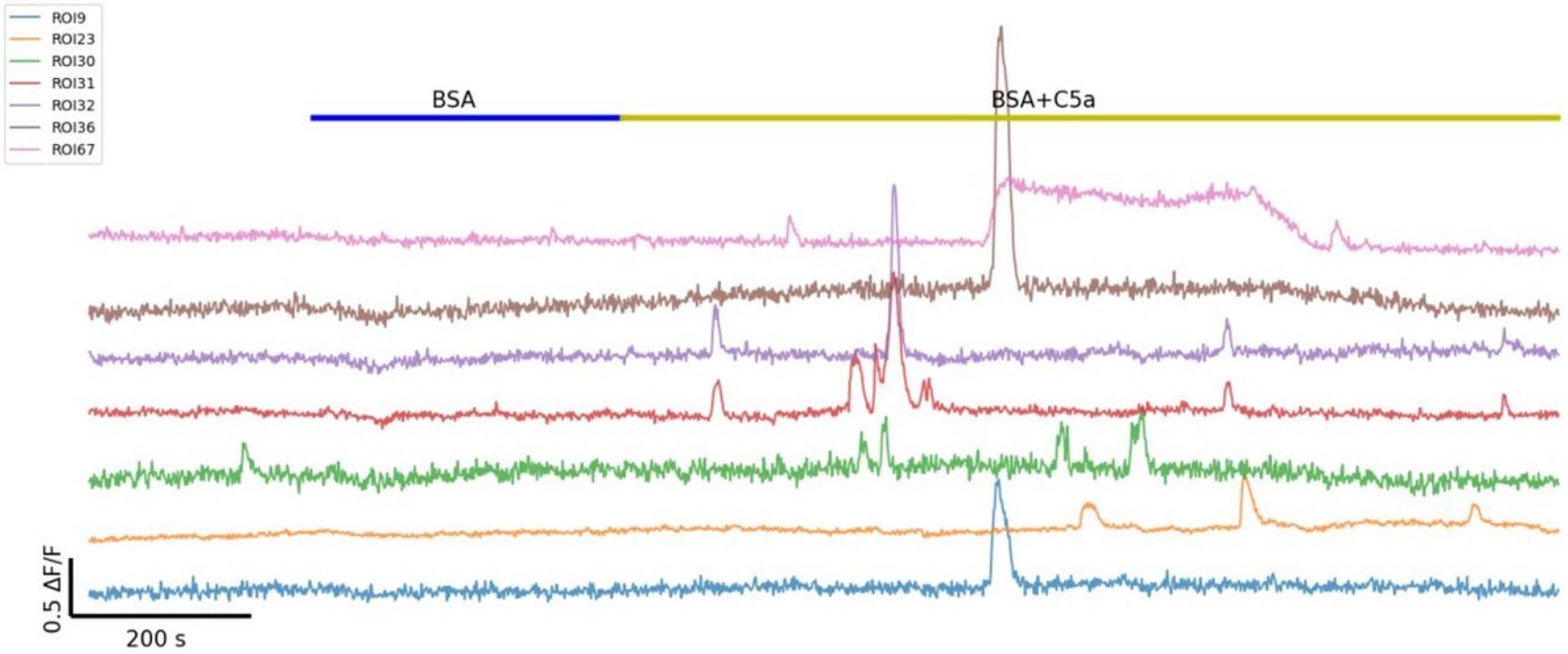
Is there some desensitization of C5aRs?

It seems that the effect of C5a is maximal at the beginning and slowly and gradually diminished. Having the time course of C5a effect on microglia we may predict a time course of possibly changes in neuronal activity.

Is the effect of C5a washable?

Most probably NO. It means we need to carry out experiments with PMX in a design when it is pre-applied before C5a application (3-5 experiments are necessary).
Possibly PMX can stop the effect of C5a (both during C5a application and after C5a application. We may check it experimentally.

What readouts of microglia activity we suggest



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	A	B	C	D	E
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2	ROI2	647	6.182424	6.825691	42.19932
3	ROI2	1470	0.539626	3.201683	1.727711
4	ROI10	1301	0.38519	5.351811	2.061466
5	ROI10	1232	0.532264	5.271084	2.805606
6	ROI10	1207	0.319488	7.401665	2.364741
7	ROI10	1320	0.309388	11.85273	3.667094
8	ROI10	1181	0.414709	6.084139	2.523148
9	ROI12	957	1.059184	25.13817	26.62594
10	ROI12	933	0.740447	7.278934	5.389663
11	ROI12	1297	0.51576	8.24647	4.253198
12	ROI12	998	0.662614	5.131905	3.400474
13	ROI12	1311	0.612693	5.947062	3.643722
14	ROI12	832	0.396104	19.00761	7.528982
15	ROI12	899	0.29057	13.08161	3.801127
16	ROI13	930	3.353547	5.348798	17.93745
17	ROI13	899	1.386385	6.78761	9.410242
18	ROI14	930	2.625928	5.780971	15.18041
19	ROI14	897	1.884301	7.052169	13.28841
20	ROI14	874	0.600153	6.534655	3.921796
21	ROI14	1451	0.467436	5.38029	2.514939
22	ROI14	653	0.41595	5.366205	2.232071
23	ROI17	896	1.032533	3.44942	3.561641
24	ROI17	798	0.648945	11.866	7.700384
25	ROI17	1288	0.40937	4.383127	1.79432
26	ROI18	1439	1.756456	7.822781	13.74037

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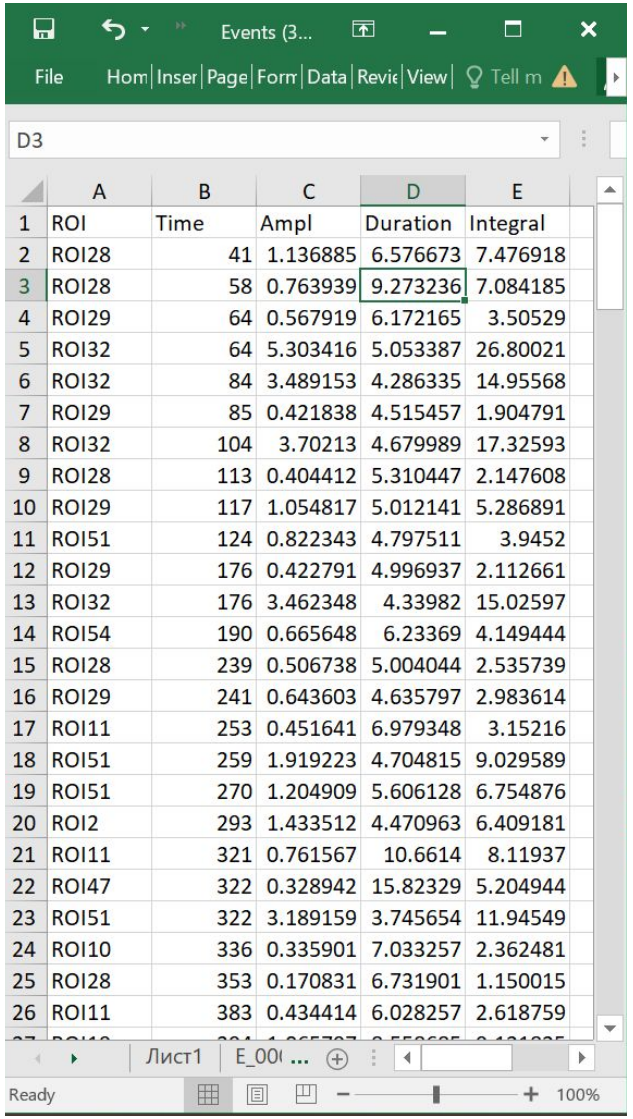
Cumulative plots for statistical comparisons and mean or median values as parameters to quantify changes



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1	ROI	Time	Ampl	Duration	Integral
2	ROI28	41	1.136885	6.576673	7.476918
3	ROI28	58	0.763939	9.273236	7.084185
4	ROI29	64	0.567919	6.172165	3.50529
5	ROI32	64	5.303416	5.053387	26.80021
6	ROI32	84	3.489153	4.286335	14.95568
7	ROI29	85	0.421838	4.515457	1.904791
8	ROI32	104	3.70213	4.679989	17.32593
9	ROI28	113	0.404412	5.310447	2.147608
10	ROI29	117	1.054817	5.012141	5.286891
11	ROI51	124	0.822343	4.797511	3.9452
12	ROI29	176	0.422791	4.996937	2.112661
13	ROI32	176	3.462348	4.33982	15.02597
14	ROI54	190	0.665648	6.23369	4.149444
15	ROI28	239	0.506738	5.004044	2.535739
16	ROI29	241	0.643603	4.635797	2.983614
17	ROI11	253	0.451641	6.979348	3.15216
18	ROI51	259	1.919223	4.704815	9.029589
19	ROI51	270	1.204909	5.606128	6.754876
20	ROI2	293	1.433512	4.470963	6.409181
21	ROI11	321	0.761567	10.6614	8.11937
22	ROI47	322	0.328942	15.82329	5.204944
23	ROI51	322	3.189159	3.745654	11.94549
24	ROI10	336	0.335901	7.033257	2.362481
25	ROI28	353	0.170831	6.731901	1.150015
26	ROI11	383	0.434414	6.028257	2.618759

Using statistics for interevent intervals, amplitudes, duration and intergrals we will be able to obtain more comprehensive analysis and dose dependence of C5aR activation.

We need to do check if we can use already performed dose-dependent experiments and process them. Or to do some additional experiments.

Norm traces E0002, before C5a