Simulated 12-well experiment: treated against control

Treated compared with Control across 12 wells. 3 of 8 measures differed between conditions after correction for multiple testing: mean firing rate (3.20 against 2.20 Hz, q 0.035); single-electrode bursts (13.34 against 7.35 per min, q 0.040); network burst rate (18.20 against 10.40 per min, q 0.035).

12	2	3 of 8	×1.75
wells analyzed	conditions	measures that differ (q < 0.05)	network burst rate, Treated vs Control

1. Design

12 wells were analyzed: 6 Control, 6 Treated. Each well was recorded for 300 seconds. Each well is treated as one independent unit in the statistics.

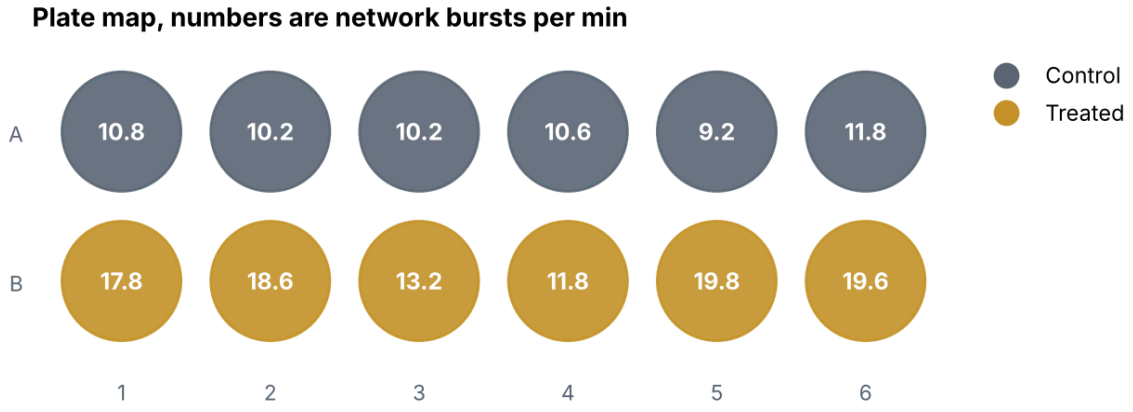


Figure 1. Wells by condition. Numbers are network bursts per minute.

2. Results by condition

3 of 8 measures differed between conditions after correction for multiple testing: mean firing rate (3.20 against 2.20 Hz, q 0.035); single-electrode bursts (13.34 against 7.35 per min, q 0.040); network burst rate (18.20 against 10.40 per min, q 0.035).

Measure	Control, median (IQR)	Treated, median (IQR)	Difference	p	q
Active electrodes	15 (15 to 15)	14 (14 to 15)	+0 (×0.97)	0.432	0.493
Mean firing rate (Hz)	2.20 (2.13 to 2.23)	3.20 (2.67 to 3.27)	+0.99 (×1.45)	0.009	**0.035**
Single-electrode bursts (per min)	7.35 (7.00 to 7.58)	13.34 (10.16 to 13.58)	+5.96 (×1.81)	0.015	**0.040**
Network burst rate (per min)	10.40 (10.20 to 10.75)	18.20 (14.35 to 19.35)	+7.70 (×1.75)	0.006	**0.035**
Network burst duration (ms)	425 (417 to 428)	422 (412 to 428)	+0 (×0.99)	1.000	1.000
Spikes in network bursts (%)	68.3 (66.7 to 69.4)	81.3 (76.9 to 82.6)	+12.8 (×1.19)	0.065	0.087
Network burst interval CV	0.33 (0.32 to 0.35)	0.36 (0.35 to 0.38)	+0.04 (×1.11)	0.065	0.087
Mean synchrony (STTC)	0.44 (0.43 to 0.44)	0.50 (0.49 to 0.51)	+0.06 (×1.13)	0.065	0.087

Difference is the Hodges-Lehmann estimate of the shift (Treated minus Control), with the ratio of medians in brackets. p from a two-sided Mann-Whitney U test. q is the Benjamini-Hochberg false discovery rate across all measures; bold q values are below 0.05.

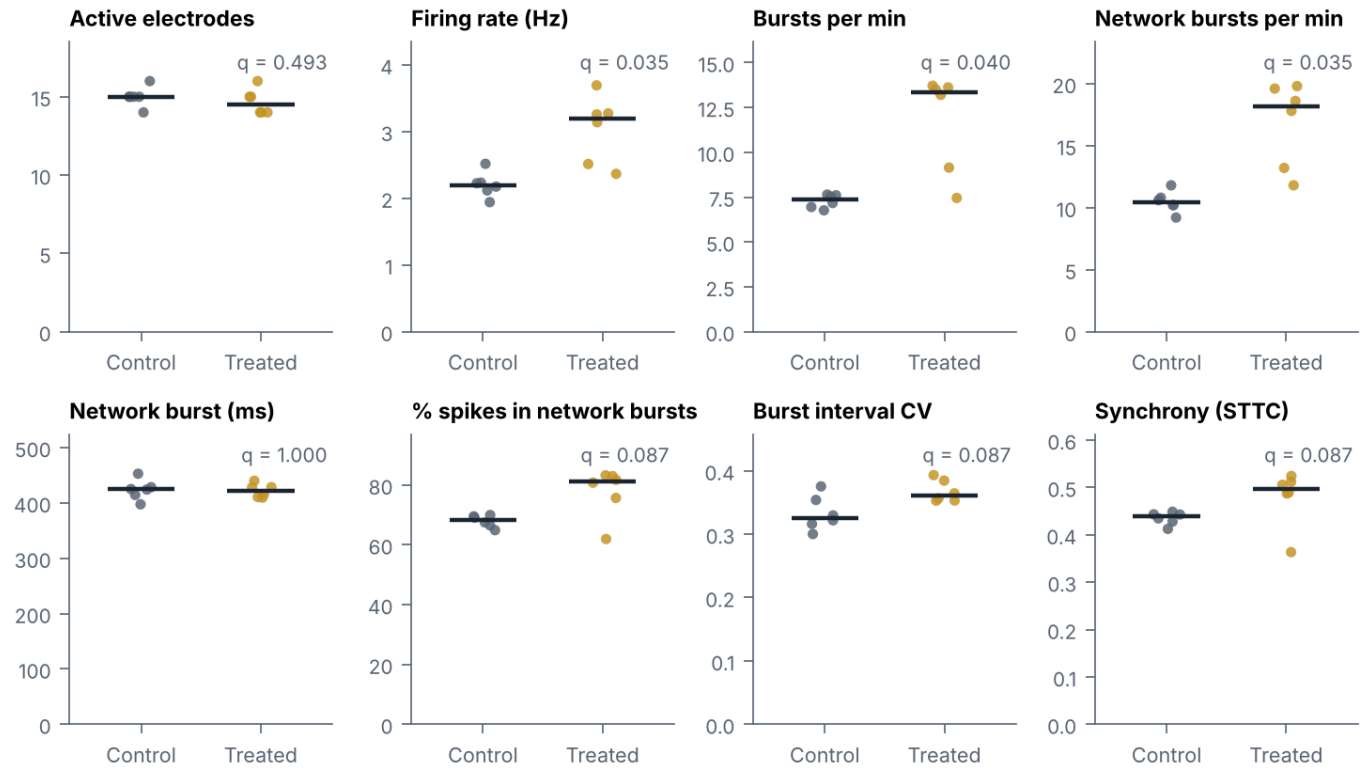


Figure 2. Each dot is one well; horizontal lines are medians. q values are corrected for testing 8 measures.

3. Robustness

We repeated the comparison under 8 alternative settings. 8 of 8 measures gave the same answer under every setting. Every significant result held under most or all settings.

Measure	Main result	Same result in	Same direction	Verdict
Active electrodes	no difference	9 of 9 settings	yes	robust
Mean firing rate	different	9 of 9 settings	yes	robust
Single-electrode bursts	different	9 of 9 settings	yes	robust
Network burst rate	different	9 of 9 settings	yes	robust
Network burst duration	no difference	9 of 9 settings	yes	robust
Spikes in network bursts	no difference	9 of 9 settings	yes	robust
Network burst interval CV	no difference	9 of 9 settings	yes	robust
Mean synchrony (STTC)	no difference	9 of 9 settings	yes	robust

Settings tried: Burst interval 50 ms; Burst interval 200 ms; Network threshold 3 MAD; Network threshold 5 MAD; Participation 25%; Participation 50%; STTC window 25 ms; STTC window 100 ms. Spike detection was not repeated.

4. Per well results

Well	Condition	Active	Rate (Hz)	NB/min	NB ms	% in NB	STTC
A1	Control	15	2.23	10.80	428	69.5	0.44
A2	Control	15	2.52	10.20	453	66.5	0.43
A3	Control	16	1.94	10.20	397	69.9	0.43
A4	Control	14	2.17	10.60	425	67.5	0.41
A5	Control	15	2.23	9.20	424	64.9	0.45
A6	Control	15	2.12	11.80	414	69.1	0.44
B1	Treated	14	3.27	17.80	429	83.0	0.52
B2	Treated	15	3.69	18.60	440	80.8	0.51
B3	Treated	14	2.51	13.20	410	75.7	0.49
B4	Treated	15	2.37	11.80	411	61.9	0.36
B5	Treated	16	3.26	19.80	416	83.2	0.51
B6	Treated	14	3.14	19.60	428	81.7	0.49

5. Methods

Spike times were analyzed per well. Electrodes firing at least 0.1 spikes per second were counted as active. Single-electrode bursts were runs of at least 5 spikes with interspike intervals of at most 100 ms. Network bursts were detected from the summed firing rate of active electrodes in 10 ms bins smoothed over 50 ms: candidate events exceeded the median plus 4 times the median absolute deviation, and were kept if at least 35% of active electrodes and 50 spikes took part. Synchrony was the spike time tiling coefficient (STTC; Cutts and Eglen, 2014) with a 50 ms window, averaged over all pairs of active electrodes. Wells with fewer than 4 active electrodes were excluded from network measures. Conditions were compared with two-sided Mann-Whitney U tests, with the

Hodges-Lehmann estimate of the difference, and p values were corrected across measures with the Benjamini-Hochberg procedure. Robustness was checked by repeating the comparison under 8 alternative settings of the burst, network burst and synchrony parameters. Analysis used mearep 0.3.1 (Wellspike), built on SpikeInterface, NumPy, SciPy and pandas.

6. Limitations

- Each well counts as one unit. If several wells come from the same differentiation batch or plate, they are not fully independent, and the p values are optimistic. Tell us the batch structure and we add it to the model.
- With 6 to 6 wells per condition, only large differences can be detected reliably. Absence of a significant difference is not evidence of no effect.
- Spike lists exported from the recording software contain no raw signal, so noise levels and detection thresholds could not be checked here.

7. Reproducibility

Every number in this report was produced by the command below. Running it again on the same input gives the same result.

```
python -m mearep experiment --input ../data/plate01/spike_list.csv --plate-map ../data/plate01/plate_map.csv --d
uration 300 --reference Control --out ../out/v031/exp01 --report-id EXP-DEMO-0001 --dataset-name 'Simulated 12-w
ell experiment: treated against control'
```

Input checksum (SHA-256): spike_list.csv 514c29bb9e446bd7; plate_map.csv 55bc08c4cd5d5875
Software: Python 3.13.18, mearep 0.3.1, SpikeInterface 0.106.0, Neo 0.14.6, NumPy 2.5.3, SciPy 1.18.1, pandas 3.0.5, NetworkX 3.7, Matplotlib 3.11.2