MEA-Based Electrophysiological Analysis of Mouse Primary Neuron Responses to Test Compounds

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Abstract

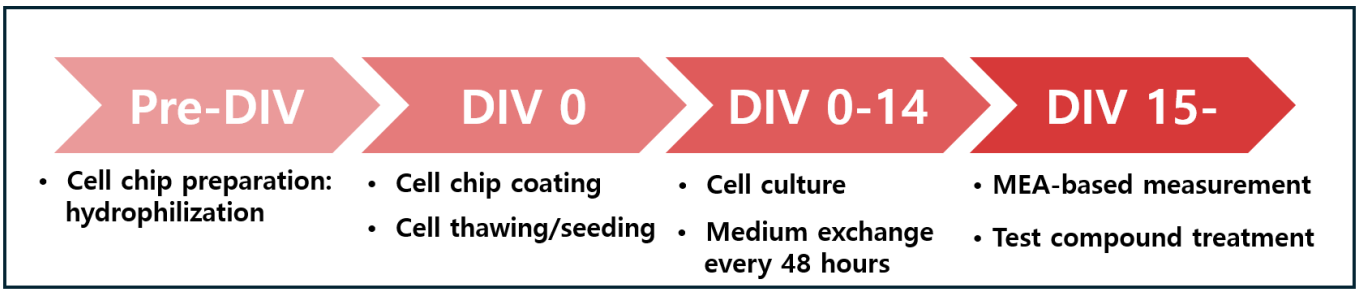
Acute and chronic electrophysiological responses of mouse primary neurons to test compounds were analyzed using the CIMFS-32 system. During the acute response period, the Non-treated group remained near baseline levels. Compared with the Non-treated group, Treatment B produced the greatest increase in neuronal firing and network activity, whereas Treatment C caused rapid suppression of electrophysiological activity immediately after treatment.

During the chronic response period, the Non-treated group showed day-to-day changes that were smaller than those observed in the treatment groups. Treatment A maintained electrophysiological activity through Day 6 before declining at Day 8. Treatment B showed an initial increase in activity followed by progressive reductions over time, whereas Treatment C showed reduced neuronal firing together with transient increases in network activity at the early time points.

Experimental Overview

Cell Type	Mouse primary neuron	**Seeding Density**	60,000 cells/well
System	CIMFS-32	**Cell Chip**	CITO-02W16E-10A
Coating Reagent	Poly-D-lysin, Laminin	**Treatment**	Three blinded test compounds (Treatment A, B, C)

Experimental Flow



Cell Chip Hydrophilization (Pre-DIV)

- Cell chips were hydrophilized one day prior to cell seeding.

Cell Preparation (DIV 0)

- Wells were coated with 50 μg/mL poly-D-lysine followed by 1 μg/mL laminin.
- Mouse primary neurons were seeded at 60,000 cells/well.

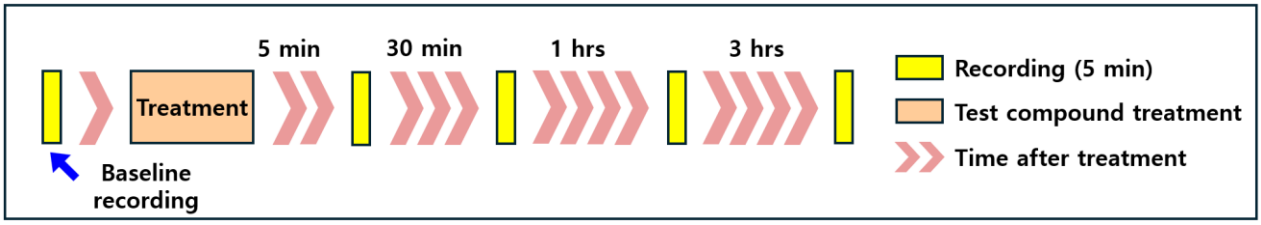
Cell Culture (DIV 0-15)

- Cells were cultured in a humidified incubator at 37°C and 5% CO_2.
- One-third of the culture medium was replaced every two days.

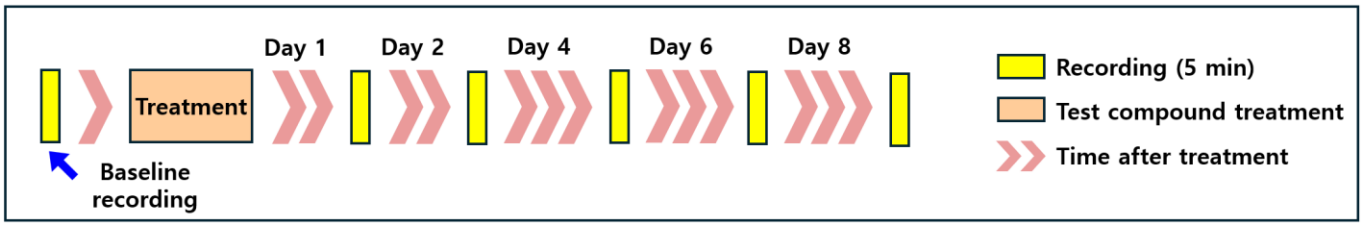
Baseline Recording and Compound Treatment (DIV 15-)

- One-third of the culture medium was replaced with fresh medium one day prior to compound treatment.
- A 5-min baseline recording was performed prior to compound treatment.
- Test compounds were added by replacing one-third of the culture medium with an equal volume of pre-warmed compound-containing medium.
- Following compound treatment, electrophysiological activity was recorded at the indicated acute and chronic assessment time points
- No additional medium exchange or compound treatment was performed during the study period.

Acute Response Testing Timeline



Chronic Response Testing Timeline



Analysis Settings

- **Signal Filtering**

  Zero-phase filtering: Enabled
  High-pass filter: Butterworth, 200 Hz, Order 2
  Low-pass filter: Butterworth, 3000 Hz, Order 2
  Notch filter: Disabled

- **Spike Detection**

  Detection method: Adaptive threshold
  Threshold: ±6 / ±7 × STD
  Minimum inter-spike interval (ISI): 2 ms
  Minimum spike rate: 0.1 spikes/min
  Initial skip time: 10 s

- **Burst Detection**

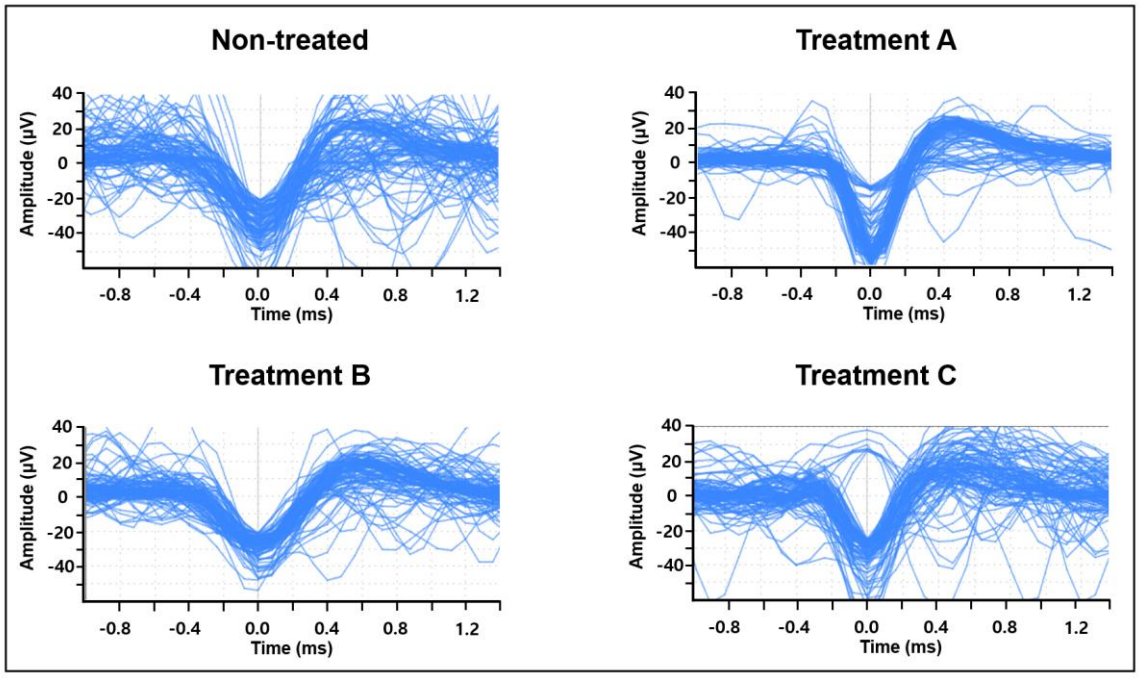
  Minimum spike count per burst: 4
  Maximum ISI within burst: 100 ms

- **Network Burst Detection**

  Network burst detection threshold: 25% of active electrodes
  Time window: 500 ms
  Maximum inter-burst interval (IBI): 100 ms

Spike Waveform

- Representative spike waveforms of each experimental group recorded at DIV 15.



Results

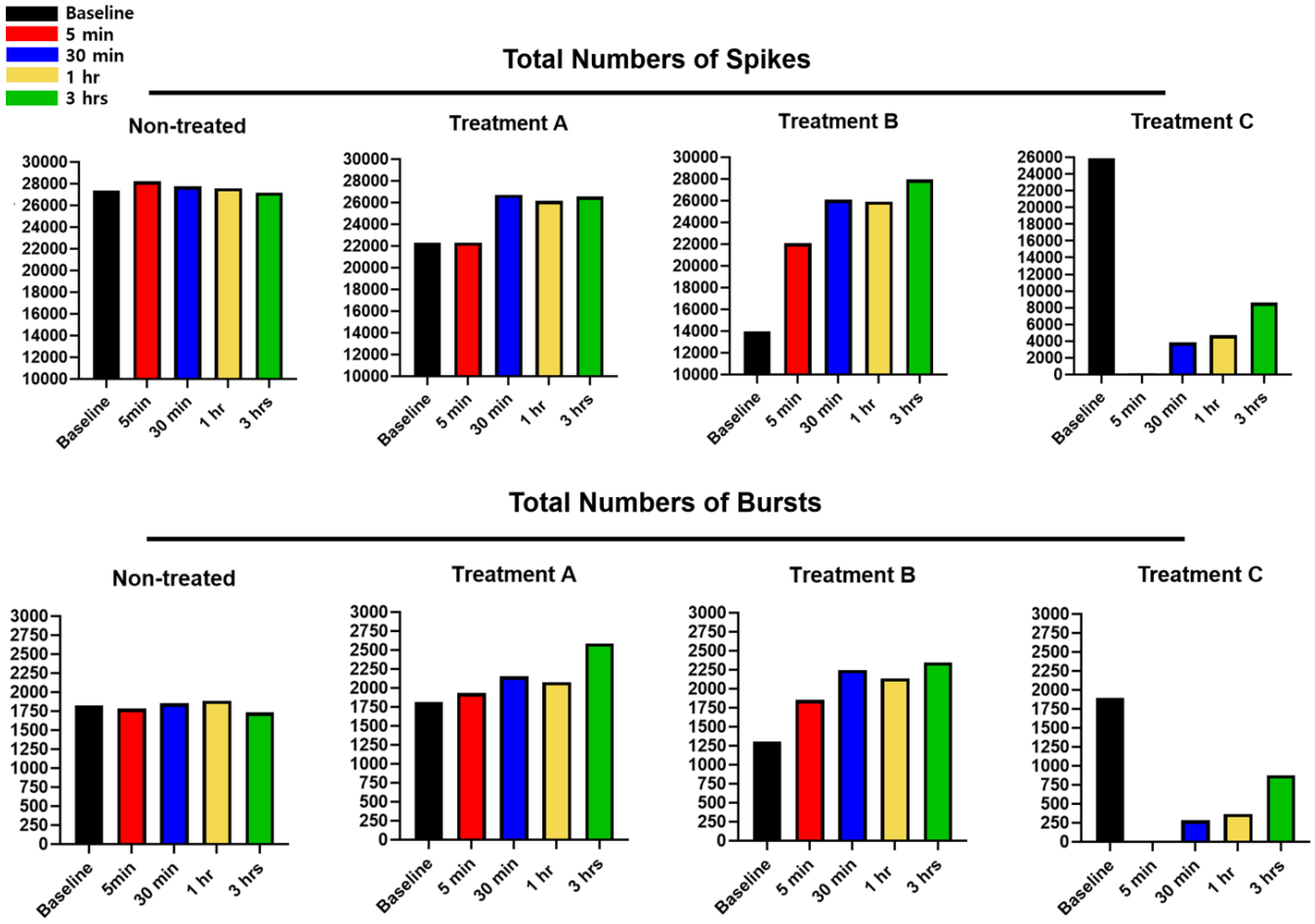


Figure 1. Changes in Spike and Burst Activity Following Test Compound Treatment (Acute Response).

To investigate acute changes in neuronal activity following compound treatment, the total number of spikes and total number of bursts were analyzed from 5-min recordings obtained at each time point.

The Non-treated group showed minimal changes in spike and burst activity following medium exchange. Total spike number remained between 27,194 and 28,208, while total burst number remained between 1,734 and 1,888.

Treatment A increased both spike and burst activity during the recording period. Total spike number increased from 22,294 at baseline to 26,534 at 3 hrs (+19.0%), while total burst number increased from 1,813 to 2,585 (+42.6%).

Treatment B produced the greatest increase in neuronal activity among all treatment groups. Total spike number increased from 13,997 at baseline to 27,940 at 3 hrs (+99.6%), while total burst number increased from 1,302 to 2,348 (+80.3%).

In contrast, Treatment C rapidly suppressed neuronal activity immediately after treatment. Total spike number decreased from 25,857 at baseline to 168 at 5 min (−99.4%), while total burst number decreased from 1,898 to 1 (−99.9%). Although both parameters increased during the subsequent recording period, neither spike nor burst activity recovered to baseline levels by 3 hrs.

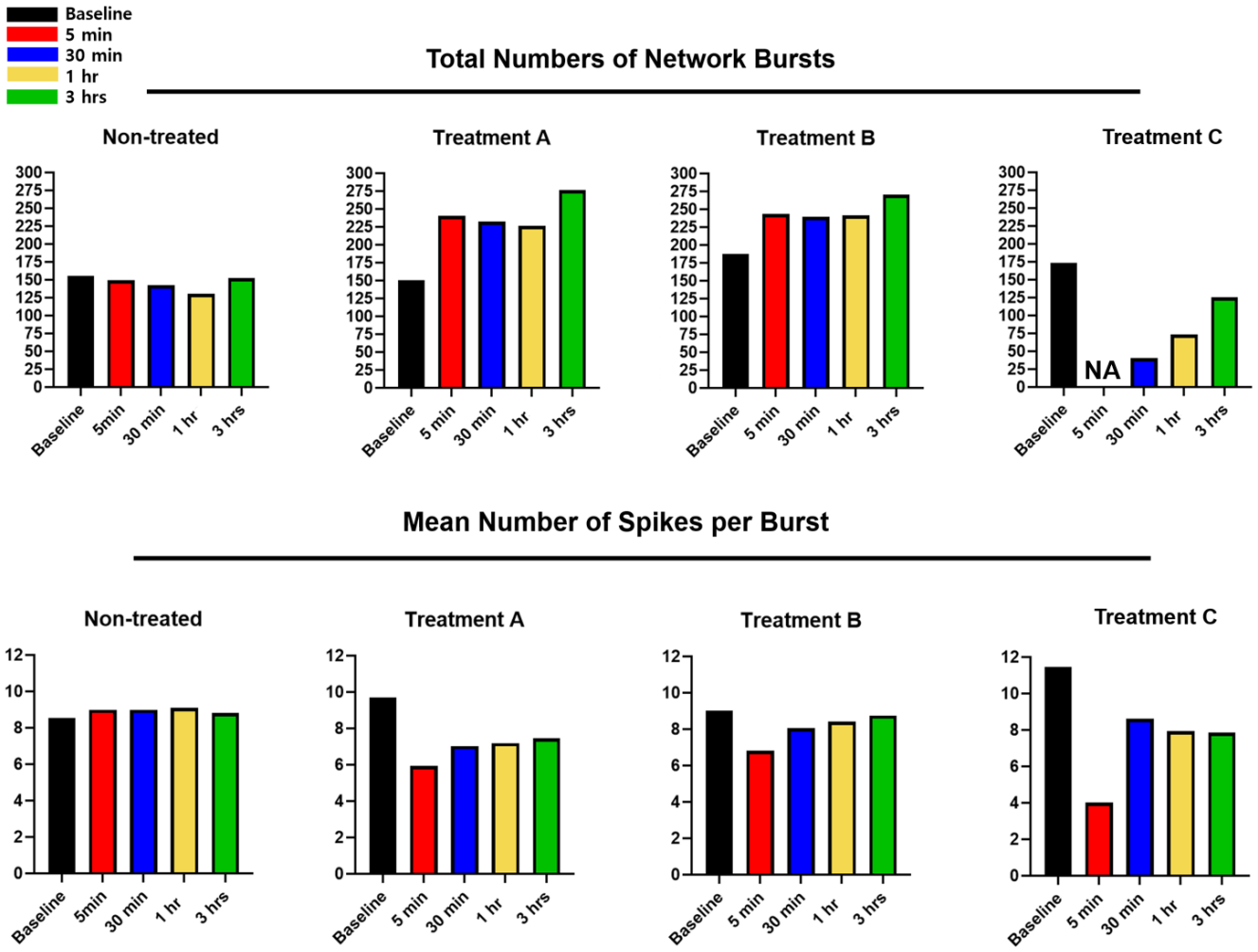


Figure 2. Changes in Network Burst Activity Following Test Compound Treatment (Acute Response).

To investigate changes in neuronal network activity following compound treatment, the total number of network bursts and mean number of spikes per burst were analyzed from 5-min recordings obtained at each time point.

The Non-treated group showed minimal changes in network burst activity following medium exchange. Total number of network bursts remained between 130 and 155, while mean number of spikes per burst remained between 8.539 and 9.089.

Treatment A increased the total number of network bursts from 150 at baseline to 276 at 3 hrs (+84.0%). In contrast, mean number of spikes per burst decreased from 9.683 at baseline to 5.933 at 5 min (−38.7%) and remained below baseline levels throughout the recording period.

Treatment B also increased network burst activity, with the total number of network bursts increasing from 187 at baseline to 270 at 3 hrs (+44.4%). Mean number of spikes per burst decreased from 9.035 to 6.817 at 5 min (−24.5%) and recovered to 8.756 by 3 hrs.

Treatment C showed the strongest suppression of network activity immediately after treatment. No network bursts were detected at 5 min. The total number of network bursts subsequently increased from 40 at 30 min to 125 at 3 hrs but remained 27.7% below the baseline value of 173. Mean number of spikes per burst decreased from 11.466 at baseline to 4.000 at 5 min (−65.1%) and partially recovered during the subsequent recording period. NA = Not Available (Network burst not detected).

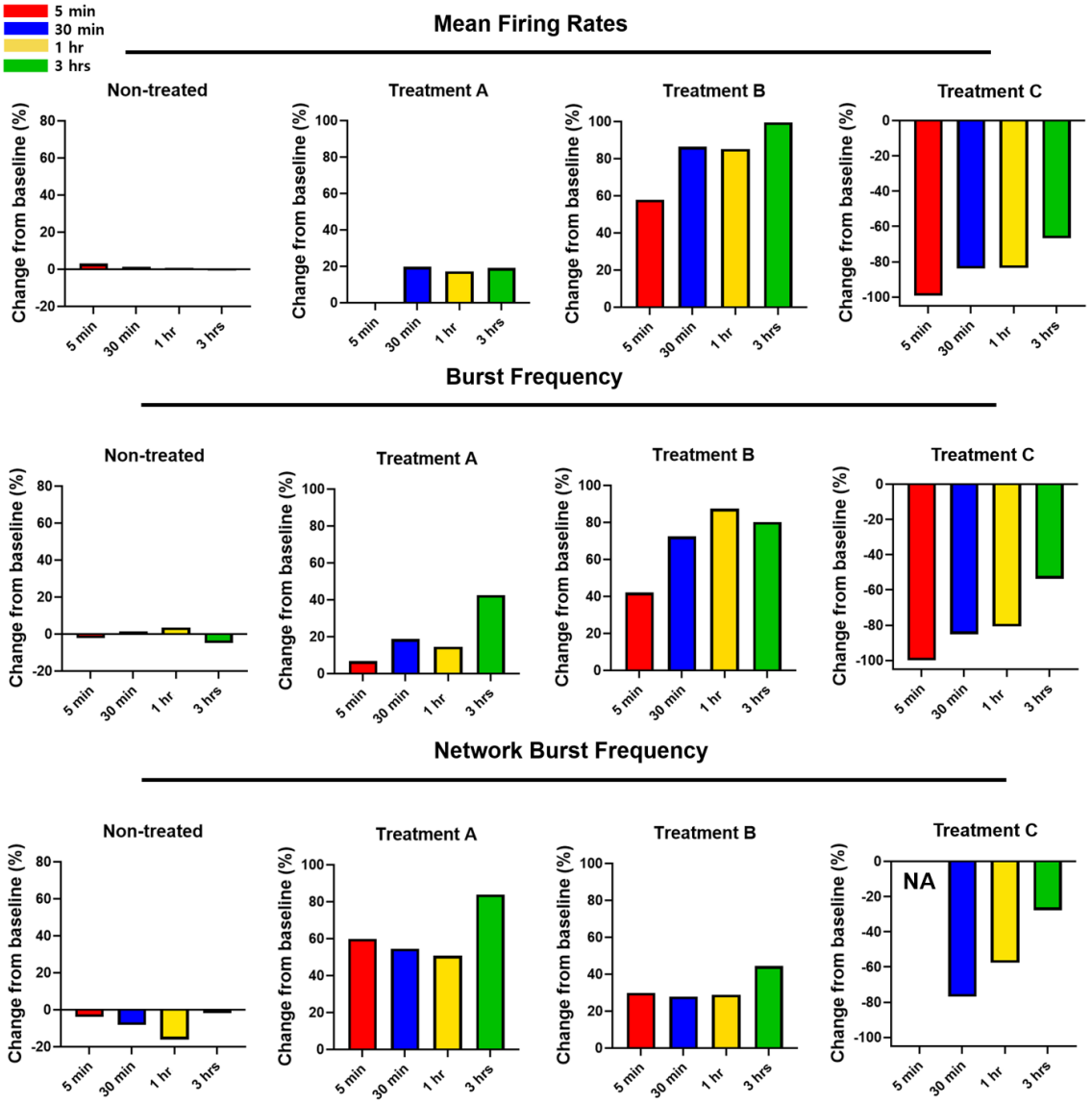


Figure 3. Changes in Key Electrophysiological Parameters Following Test Compound Treatment (Acute Response).

Changes in Mean Firing Rate (MFR), Burst Frequency (BF), and Network Burst Frequency (NBF) were analyzed as percentage changes from baseline following compound treatment.

The Non-treated group showed small changes in MFR, BF, and NBF throughout the acute response period. MFR ranged from −0.624% to +3.088%, BF from −4.887% to +3.566%, and NBF from −16.105% to −1.873%.

Treatment A primarily affected network-level activity. MFR increased by up to 19.777%, while BF increased by up to 42.596%. NBF increased at all time points, ranging from +50.677% to +83.946%.

Treatment B produced the greatest increase in neuronal firing and burst activity. MFR increased from +57.850% at 5 min to +99.627% at 3 hrs, while BF increased from +42.077% to +80.321%. NBF also increased throughout the recording period, reaching +44.410% at 3 hrs.

In contrast, Treatment C produced the greatest reduction in electrophysiological activity. MFR decreased by 99.343% at 5 min and remained 66.793% below baseline at 3 hrs. BF decreased by 99.954% at 5 min and remained 53.685% below baseline at 3 hrs. No network bursts were detected at 5 min, and NBF remained below baseline levels throughout the recording period, ranging from −76.846% to −27.685%. NA = Not Available (Network burst not detected).

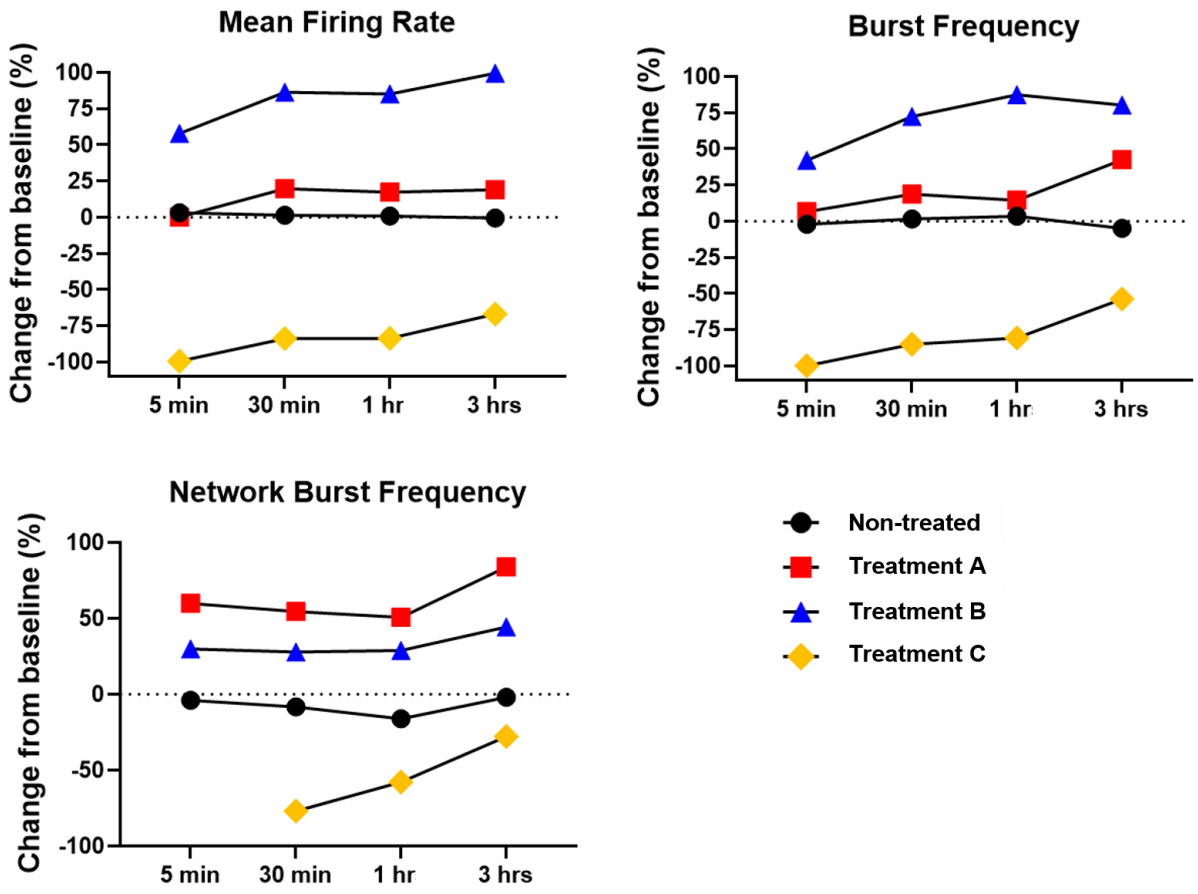


Figure 4. Comparison of Changes in Key Electrophysiological Parameters Following Test Compound Treatment (Acute Response).

Changes in Mean Firing Rate (MFR), Burst Frequency (BF), and Network Burst Frequency (NBF) were compared among treatment groups using change from baseline (%).

The Non-treated group remained near baseline levels during the acute response period. Compared with the Non-treated group, Treatment A increased MFR, BF, and NBF, with the largest change observed in NBF. Treatment B showed the greatest increases in MFR, BF, and NBF among all treatment groups, indicating enhanced neuronal firing and network activity.

In contrast, Treatment C markedly reduced MFR, BF, and NBF and showed no detectable network bursts at 5 min. Although electrophysiological activity partially recovered during the subsequent recording period, all parameters remained below baseline levels at 3 hrs.

Overall, Treatment A primarily enhanced network activity, Treatment B increased both neuronal firing and network activity, whereas Treatment C suppressed electrophysiological activity compared with the Non-treated group.

Table 1. Electrophysiological parameter analysis (Acute response after treatment)

Non-treated

Parameters	Baseline	Time after treatment			
		5 min	30 min	1 hrs	3 hrs
Active Channel	14	14	14	14	14
Total Number of Spikes [spks]	27,362	28,208	27,737	27,579	27,194
Number of Spikes per Channel [spks]	1,954	2,015	1,981	1,970	1,942
Mean Firing Rate [spk/s, (Hz)]	6.735	6.943	6.827	6.788	6.693
Total Number of Bursts [brts]	1,823	1,783	1,850	1,888	1,734
Number of Bursts per Channel [brts]	130.214	127.357	132.143	134.857	123.857
Burst Frequency [Hz]	6.282	6.144	6.375	6.506	5.975
Mean Number of Spikes per Burst [spks]	8.539	8.992	8.988	9.089	8.809
Number of Network Bursts	155.000	149.000	142.000	130.000	152.000
Network Bursts Frequency [Hz]	0.534	0.513	0.490	0.448	0.524

Treatment A

Parameters	Baseline	Time after treatment			
		5 min	30 min	1 hrs	3 hrs
Active Channel	15	15	15	15	15
Total Number of Spikes [spks]	22,294	22,321	26,706	26,159	26,534
Number of Spikes per Channel [spks]	1,486	1,488	1,780	1,744	1,769
Mean Firing Rate [spk/s, (Hz)]	5.122	5.128	6.135	6.009	6.096
Total Number of Bursts [brts]	1,813	1,934	2,153	2,077	2,585
Number of Bursts per Channel [brts]	120.867	128.933	143.533	138.467	172.333
Burst Frequency [Hz]	6.247	6.664	7.419	7.157	8.908
Mean Number of Spikes per Burst [spks]	9.683	5.933	7.023	7.182	7.447
Number of Network Bursts	150.000	240.000	232.000	226.000	276.000
Network Bursts Frequency [Hz]	0.517	0.827	0.799	0.779	0.951

Treatment B

Parameters	Baseline	Time after treatment			
		5 min	30 min	1 hrs	3 hrs
Active Channel	12	12	12	12	12
Total Number of Spikes [spks]	13,997	22,091	26,088	25,925	27,940
Number of Spikes per Channel [spks]	1,166	1,841	2,174	2,160	2,328
Mean Firing Rate [spk/s, (Hz)]	4.019	6.344	7.491	7.445	8.023
Total Number of Bursts [brts]	1,302	1,850	2,245	2,137	2,348
Number of Bursts per Channel [brts]	108.500	154.167	187.083	178.083	195.667
Burst Frequency [Hz]	4.487	6.375	7.736	8.412	8.091
Mean Number of Spikes per Burst [spks]	9.035	6.817	8.049	8.412	8.756
Number of Network Bursts	187.000	243.000	239.000	241.000	270.000
Network Bursts Frequency [Hz]	0.644	0.837	0.824	0.830	0.930

Treatment C

Parameters	Baseline	Time after treatment			
		5 min	30 min	1 hrs	3 hrs
Active Channel	13	13	13	13	13
Total Number of Spikes [spks]	25,857	168	3,821	4,709	8,587
Number of Spikes per Channel [spks]	1,989	13	294	362	661
Mean Firing Rate [spk/s, (Hz)]	6.854	0.045	1.103	1.125	2.276
Total Number of Bursts [brts]	1,898	1	283	365	879
Number of Bursts per Channel [brts]	146.000	0.077	21.769	28.077	67.615
Burst Frequency [Hz]	6.540	0.003	0.975	1.258	3.029
Mean Number of Spikes per Burst [spks]	11.466	4.000	8.611	7.921	7.848
Number of Network Bursts	173.000	0.000	40.000	73.000	125.000
Network Bursts Frequency [Hz]	0.596	0.000	0.138	0.252	0.431

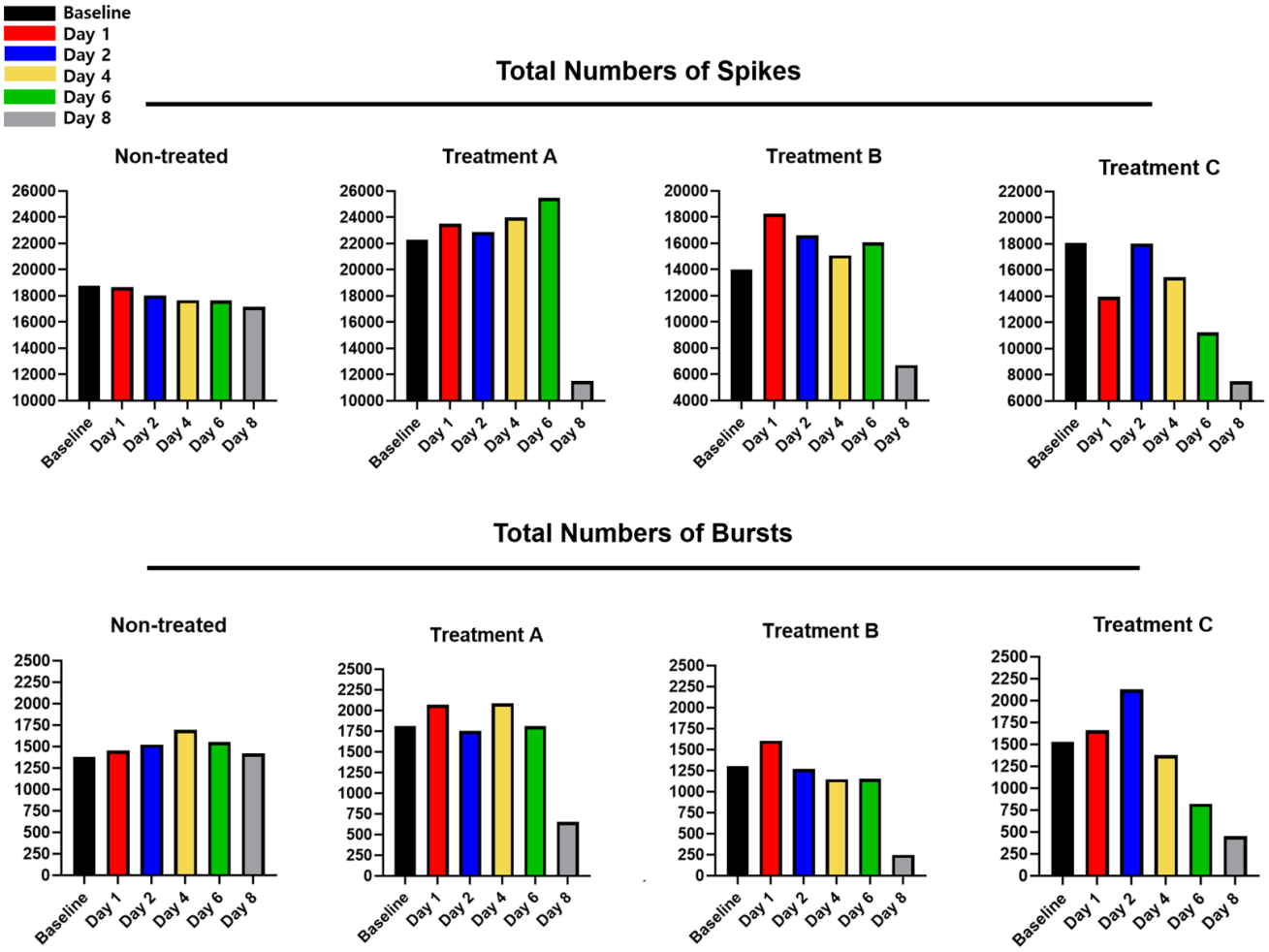


Figure 5. Changes in Spike and Burst Activity Following Test Compound Treatment (Chronic Response).

To investigate long-term changes in neuronal activity following compound treatment, the total number of spikes and total number of bursts were analyzed from 5-min recordings obtained at each time point.

The Non-treated group showed relatively small changes in spike and burst activity during the chronic response period. Total spike number decreased from 18,798 at baseline to 17,147 at Day 8 (−8.783%), while total burst number remained within ±10% of baseline throughout most of the recording period.

Treatment A maintained spike and burst activity through Day 6. Total spike number increased from 22,294 at baseline to 25,470 at Day 6 (+14.246%) before decreasing to 11,502 at Day 8 (−48.408%). Total burst number followed a similar pattern and decreased by 63.927% at Day 8.

Treatment B showed an initial increase in neuronal activity followed by progressive reductions over time. Total spike number increased by 30.421% at Day 1 but decreased by 51.997% at Day 8. Total burst number decreased by 80.952% at Day 8.

Treatment C showed a transient increase in burst activity at Day 2, followed by progressive reductions at later time points. Total spike number decreased by 58.480% and total burst number decreased by 70.419% at Day 8.

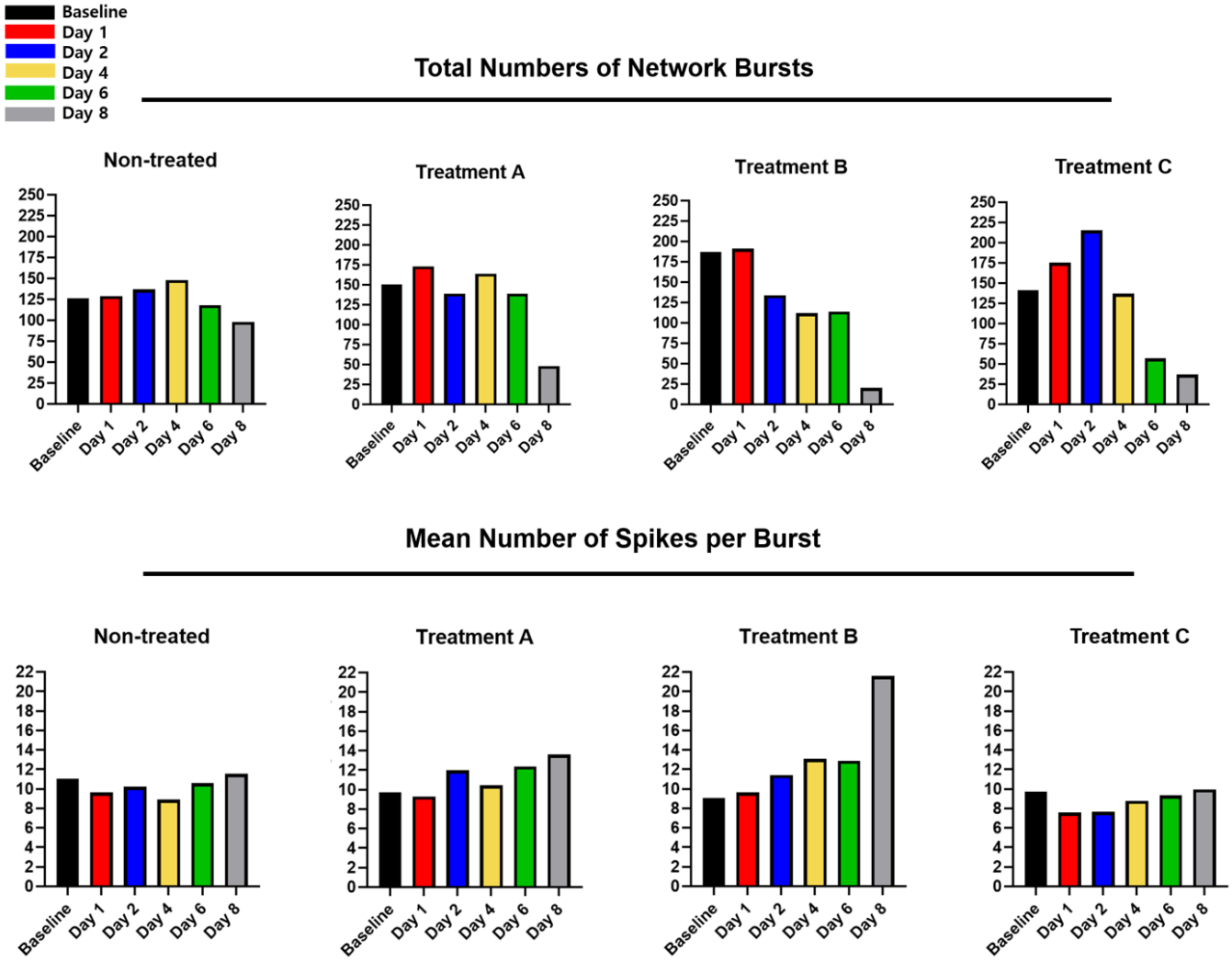


Figure 6. Changes in Network Burst Activity Following Test Compound Treatment (Chronic Response).

To investigate long-term changes in neuronal network activity following compound treatment, the total number of network bursts and mean number of spikes per burst were analyzed from 5-min recordings obtained at each time point.

The Non-treated group showed day-to-day variation in network burst activity, with the total number of network bursts ranging from 98 to 148 throughout the recording period. Mean number of spikes per burst remained between 8.879 and 11.515.

Treatment A maintained network burst activity through Day 6 before decreasing from 150 at baseline to 48 at Day 8 (−68.000%). In contrast, mean number of spikes per burst increased from 9.683 at baseline to 13.592 at Day 8 (+40.370%).

Treatment B showed progressive reductions in network burst activity, decreasing from 187 at baseline to 20 at Day 8 (−89.305%). Mean number of spikes per burst increased from 9.035 to 21.589 (+138.949%) during the same period.

Treatment C showed increased network burst activity at Day 1 and Day 2, reaching 215 network bursts at Day 2 (+52.482%), followed by marked reductions at later time points. Mean number of spikes per burst remained close to baseline throughout the recording period and increased by 2.305% at Day 8.

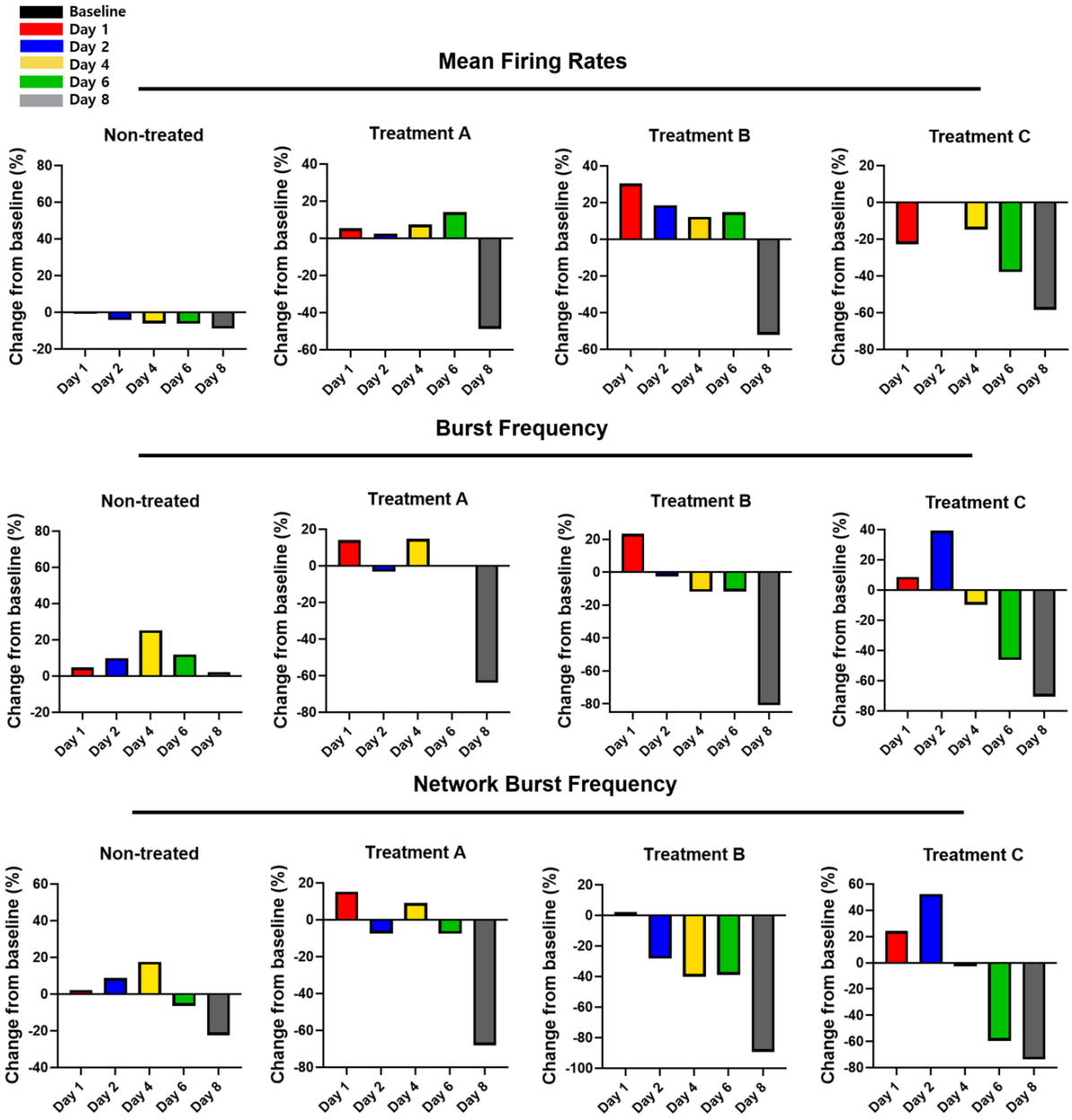


Figure 7. Changes in Key Electrophysiological Parameters Following Test Compound Treatment (Chronic Response).

Changes in Mean Firing Rate (MFR), Burst Frequency (BF), and Network Burst Frequency (NBF) were analyzed as percentage changes from baseline during the chronic response period.

The Non-treated group showed day-to-day variation in MFR, BF, and NBF without marked deviations from baseline. MFR remained within −8.904% of baseline, whereas BF and NBF reached maximum increases of 25.199% and 17.512%, respectively.

Treatment A maintained MFR above baseline through Day 6, reaching a maximum increase of 14.077%, before decreasing by 48.770% at Day 8. BF and NBF showed a similar pattern and decreased by 63.919% and 68.085%, respectively, at Day 8.

Treatment B showed the largest increase in MFR at Day 1 (+30.430%), followed by progressive reductions in BF and NBF over time. By Day 8, MFR, BF, and NBF decreased by 52.003%, 80.945%, and 89.286%, respectively.

Treatment C showed reduced MFR throughout most of the recording period, while BF and NBF increased at early time points and reached maximum increases of 39.335% and 52.469% at Day 2. However, all parameters decreased below baseline levels by Day 8.

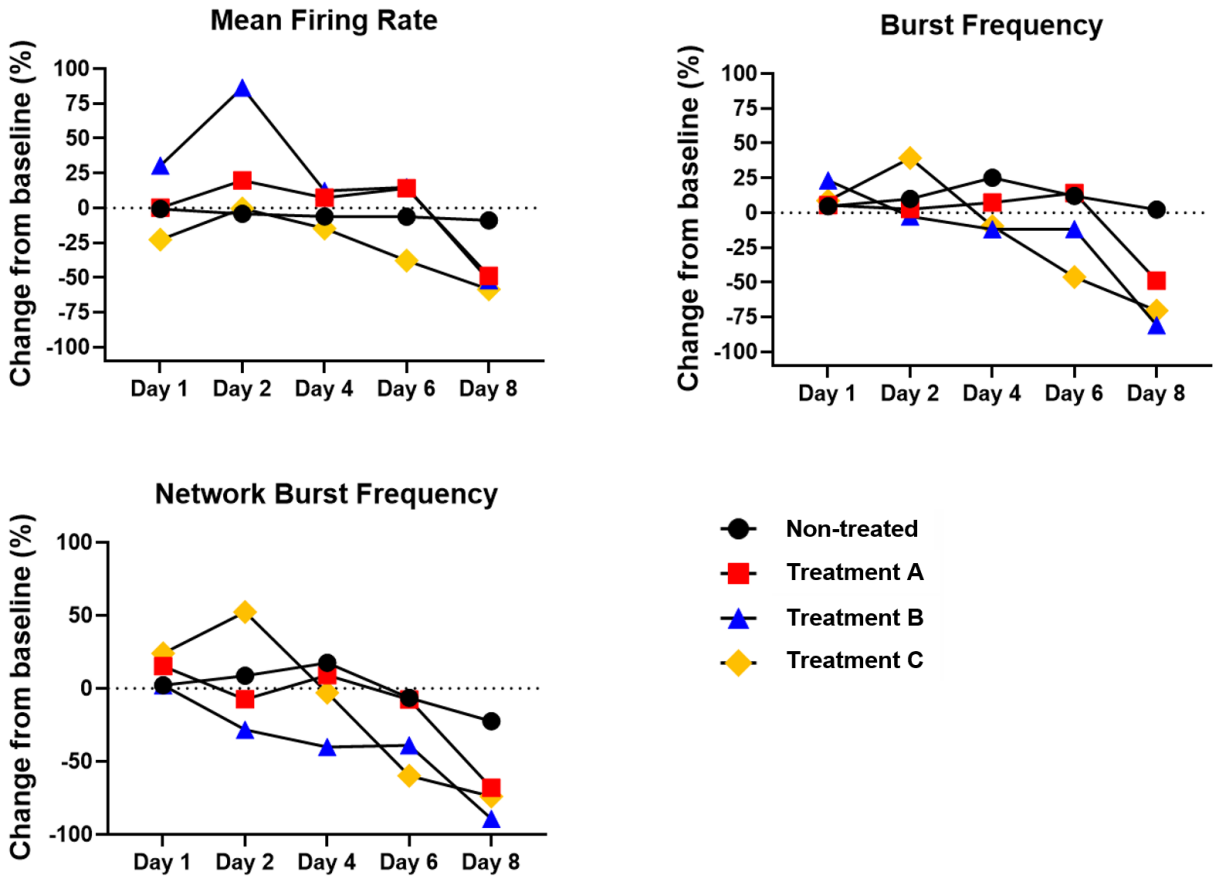


Figure 8. Comparison of Changes in Key Electrophysiological Parameters Following Test Compound Treatment (Chronic Response).

Changes in Mean Firing Rate (MFR), Burst Frequency (BF), and Network Burst Frequency (NBF) were compared among treatment groups using change from baseline (%).

Compared with the Non-treated group, Treatment A maintained electrophysiological activity through Day 6. MFR remained above baseline during the recording period until Day 6, while BF and NBF showed fluctuations around baseline before decreasing at Day 8.

Treatment B showed the largest increase in MFR at the early time points, followed by progressive reductions in BF and NBF. By Day 8, all electrophysiological parameters were reduced below baseline levels.

Treatment C showed a distinct response pattern from Treatment B. MFR remained below baseline throughout most of the recording period, whereas BF and NBF increased at the early time points before declining at later time points.

Overall, the Non-treated group showed smaller day-to-day changes than the treatment groups. Treatment A maintained electrophysiological activity through Day 6, Treatment B produced an initial increase followed by progressive reductions, and Treatment C showed transient increases in network activity despite reduced neuronal firing.

Table 2. Electrophysiological parameter analysis (Chronic response after treatment)

Non-treated

Parameters	Baseline	Time after treatment				
		Day 1	Day 2	Day 4	Day 6	Day 8
Active Channel	14	14	14	14	14	14
Total Number of Spikes [spks]	18,798	18,662	18,024	17,669	17,640	17,147
Number of Spikes per Channel [spks]	1,343	1,333	1,287	1,262	1,260	1,225
Mean Firing Rate [spk/s, (Hz)]	4.627	4.590	4.433	4.346	4.336	4.215
Total Number of Bursts [brts]	1,383	1,450	1,521	1,692	1,551	1,417
Number of Bursts per Channel [brts]	98.786	103.571	108.643	120.857	110.786	101.214
Burst Frequency [Hz]	4.766	4.993	5.238	5.967	5.337	4.876
Mean Number of Spikes per Burst [spks]	11.030	9.626	10.229	8.879	10.585	11.515
Number of Network Bursts	126.000	129.000	137.000	148.000	118.000	98.000
Network Bursts Frequency [Hz]	0.434	0.444	0.472	0.510	0.406	0.337

Treatment A

Parameters	Baseline	Time after treatment				
		Day 1	Day 2	Day 4	Day 6	Day 8
Active Channel	15	15	15	15	15	15
Total Number of Spikes [spks]	22,294	23,500	22,877	23,984	25,470	11,502
Number of Spikes per Channel [spks]	1,486	1,567	1,525	1,599	1,698	767
Mean Firing Rate [spk/s, (Hz)]	5.122	5.399	5.255	5.502	5.843	2.624
Total Number of Bursts [brts]	1,813	2,069	1,753	2,084	1,810	654
Number of Bursts per Channel [brts]	120.867	137.933	116.867	138.933	120.667	43.600
Burst Frequency [Hz]	6.247	7.130	6.041	7.171	6.228	2.254
Mean Number of Spikes per Burst [spks]	9.683	9.277	11.971	10.423	12.362	13.592
Number of Network Bursts	150.000	173.000	139.000	164.000	139.000	48.000
Network Bursts Frequency [Hz]	0.517	0.596	0.479	0.564	0.478	0.165

Treatment B

Parameters	Baseline	Time after treatment				
		Day 1	Day 2	Day 4	Day 6	Day 8
Active Channel	12	12	12	12	12	12
Total Number of Spikes [spks]	13,997	18,255	16,606	15,079	16,069	6,719
Number of Spikes per Channel [spks]	1,166	1,521	1,384	1,257	1,339	560
Mean Firing Rate [spk/s, (Hz)]	4.019	5.242	4.769	4.511	4.614	1.929
Total Number of Bursts [brts]	1,302	1,606	1,268	1,147	1,150	248
Number of Bursts per Channel [brts]	108.500	133.833	105.667	95.583	95.833	20.667
Burst Frequency [Hz]	4.487	5.534	4.369	3.952	3.963	0.855
Mean Number of Spikes per Burst [spks]	9.035	9.623	11.438	13.061	12.903	21.589
Number of Network Bursts	187.000	191.000	134.000	112.000	114.000	20.000
Network Bursts Frequency [Hz]	0.644	0.658	0.462	0.386	0.393	0.069

Treatment C

Parameters	Baseline	Time after treatment				
		Day 1	Day 2	Day 4	Day 6	Day 8
Active Channel	13	13	13	13	13	13
Total Number of Spikes [spks]	18,078	13,973	18,022	15,441	11,239	7,506
Number of Spikes per Channel [spks]	1,391	1,075	1,386	1,188	865	577
Mean Firing Rate [spk/s, (Hz)]	4.792	3.704	4.777	4.085	2.979	1.990
Total Number of Bursts [brts]	1,528	1,662	2,129	1,377	821	452
Number of Bursts per Channel [brts]	117.538	127.846	163.769	105.923	63.154	34.769
Burst Frequency [Hz]	5.265	5.727	7.336	4.757	2.829	1.558
Mean Number of Spikes per Burst [spks]	9.716	7.571	7.628	8.784	9.347	9.940
Number of Network Bursts	141.000	175.000	215.000	137.000	57.000	37.000
Network Bursts Frequency [Hz]	0.486	0.603	0.741	0.472	0.196	0.127

Summary

The Non-treated group maintained electrophysiological activity near baseline levels during the acute response period and showed smaller day-to-day changes than the treatment groups during the chronic response period.

Treatment A increased network activity during the acute response period and maintained neuronal and network activity through Day 6 during the chronic response period. However, electrophysiological activity declined at Day 8.

Treatment B produced the strongest increase in neuronal firing, burst activity, and network activity during the acute response period. During the chronic response period, neuronal firing increased at the early time points, followed by progressive reductions in network activity and overall electrophysiological activity.

Treatment C caused rapid suppression of neuronal firing and network activity immediately after treatment. During the chronic response period, neuronal firing remained below baseline levels, whereas network activity showed transient increases at the early time points before declining at later time points.

Supplemental Data

- No supplementary data.

Keyword

CIMFS-32, MEA, Mouse Primary Neuron, Electrophysiology, Neuronal Firing Activity, Neural Network Function

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