Supplementary materials:

BiœmuS: A new tool for neurological disorders studies through real-time emulation and hybridization using biomimetic Spiking Neural Network

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This PDF includes

- Material and methods
- Figures S1 to S8
- Tables S1 to S3
- Captions for Movies S1

Other Supplementary Materials for this manuscript include the following

- Movie S1

1 Supplementary Methods

Method S1

Central Pattern Generator as a small network emulation on smaller hardware

Another application designed explores the real-time emulation on a smaller embedded target to develop bioinspired applications. It corresponds to an energy-efficient and reduced version of BiœmuS targeting a small network that reproduces a Central Pattern Generator (CPG) as described in [1]. The hardware controls a robot mimicking snake motion [2] as shown in Figure S8A. The original Izhikevich neuron model in [2] is replaced by the SNN of BiœmuS using HH model to improve biological coherence. The reduced version of BiœmuS implementing fixed point coding and approximated ionic channel equations from [3]. Each neuron of the network is assigned to a motor to create snake alike motion based on the activity of the network.

Method S2

Closed-loop biohybrid experiment integrating an embedded version of BiœmuS

A closed-loop biohybrid experiment demonstrating connection between connectoid cortical organoids [4, 5] and the reduced version of BiœmuS presented in Method S1 was established as a proof of feasibility. It aims to mimic artificially the interaction between the brain and spinal cord (see Figure S9A). The connected organoids were plated on MEA and 1 µl of AAV-CAG-hCHR2-tdTomato (VectorBuilder Inc.) was added to the culture media at day 60 experiment for optogenetic interventions as in [6]. The experiment was performed at day 110.

Spiking activity from left and right organoids stimulates the first CPG. Sensory

feedback is introduced through optogenetic stimulation of the left organoid triggered accordingly to infrared sensors information provided by the robot that is mapped as a square area for each sensor.

MEA signals are recorded by AlphaMED64 system and forwarded to a (Red-Pitaya STEMlab125-1) performing digitalization and threshold based spike detection. Spiking information is then sent through WiFi to the snake robot. The CPG is configured on the snake robot [2] and featured with a WiFi module to implement stimulation based on the spike activity sent by the culture. Infrared sensors placed at the front of the robot were mapped to stimulation area to illuminate using DMD (see Figure S9A,D). The sequence of illumination patterns was designed using Polygon software.

On reception of the information of sensors, the RedPiataya selects the corresponding pattern on the DMD. The selection of the pattern is done using the trigger in input that allows to switch patterns from pulses. Hence the appropriate number of pulses were sent based on IR sensor information to select the matching pattern.

In this setup, the latency between spike detection from the organoids and stimulation on the snake robot is between 5-10 ms and about the same from the CPG to the organoids that corresponds to WiFi latency. This experiment promotes the use of BiœmuS as artificial neural network mimicking a specific biological network in a biohybrid experiment, here a model of the spinal cord.

2 Supplementary Figures

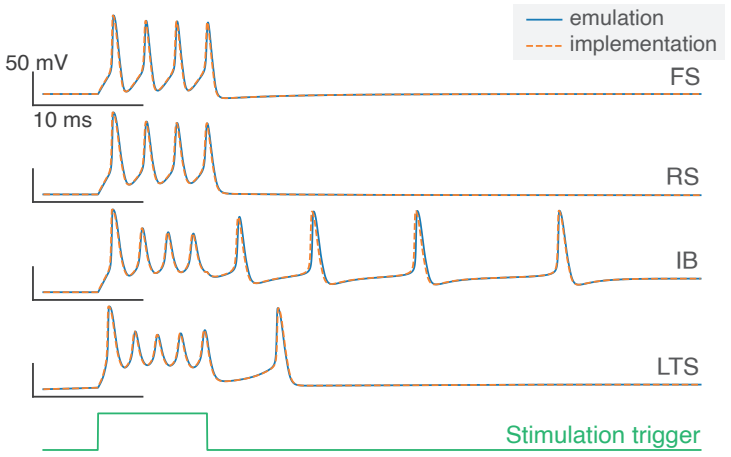

Fig. S1 Comparison of membrane voltage response in implementation to a 10 ms current injection for Fast Spiking (FS), Regular Spiking (RS), Intrinsic Bursting (IB) and Low-Threshold Spiking (LTS) neurons. Waveforms were captured using the on-board saving.

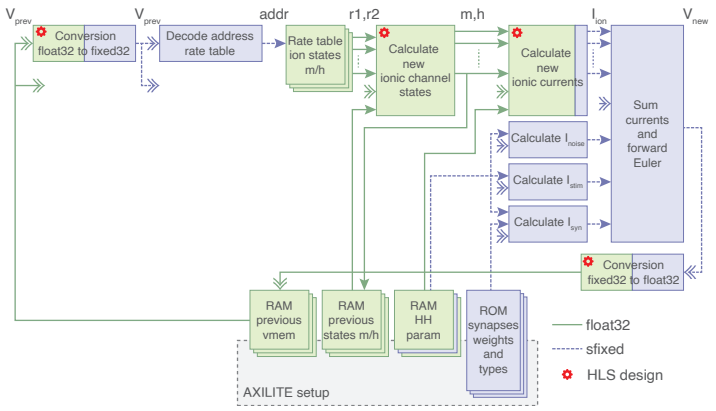

Fig. S2 Block diagram of the calculation core architecture. The data encoding, floating or fixed point, is shown for each module. Modules using floating point operations were designed using Vitis HLS. Initialization of the RAM and ROM is performed by the C++ application through AXI LITE.

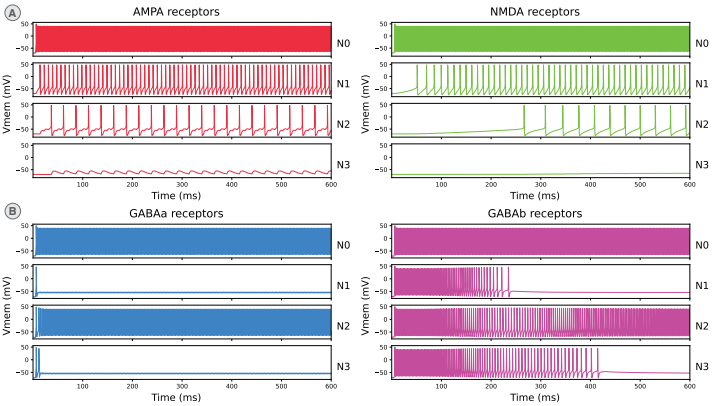

Fig. S3 Behavior of a network of 4 identical neurons where only the type of synapses changes. The network connectivity is a chaser: neurons are interconnected with a single synapse, from N0 to N1, N1 to N2 and N2 to N3. A factor is applied to synaptic weights in order to see an excitation or an inhibition despite having only one incoming synapse on each neuron. **(A)** Only N0 is stimulated: AMPAR shows a faster response of excitation than NMDAR, synaptic weight of N2 to N3 is too small resulting in a lack of excitation of N3 thus preventing spiking. **(B)** All 4 neurons are stimulated: $GABA_AR$ shows a faster response of inhibition than $GABA_BR$.

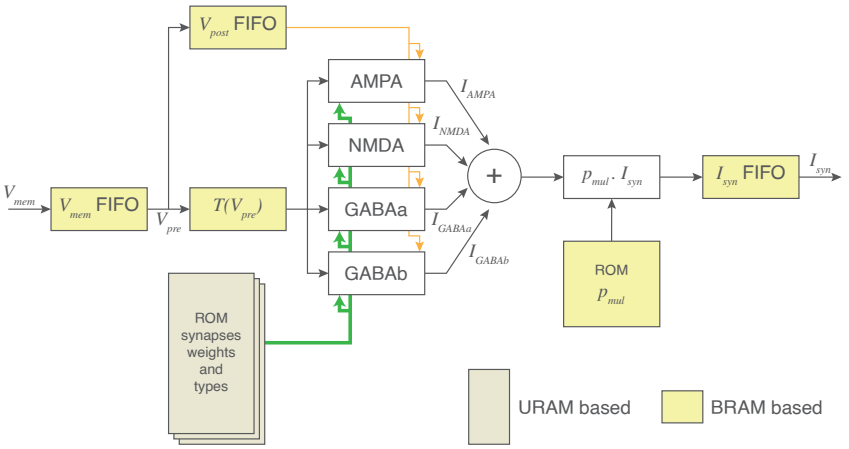

Fig. S4 Block diagram of the synaptic current calculation module using fixed point coding. The type of memory implemented is shown for blocks integrating FIFOs, ROM or rate tables. The p_{mul} ROM corresponds to premultiplied coefficients including a scale factor to mimic larger network through stronger synaptic current.

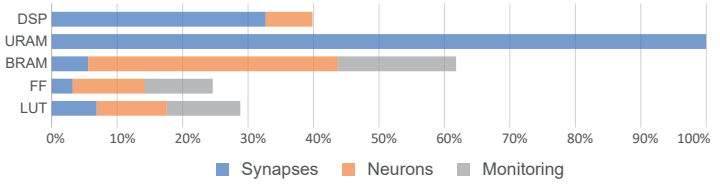

Fig. S5 Distributed resource utilization for implementation on AMD Xilinx KR260 Robotic Starter Kit exported from Vivado 2022.2.

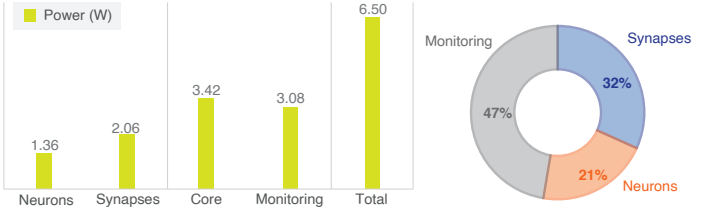

Fig. S6 Distributed power consumption for implementation on AMD Xilinx KR260 Robotic Starter Kit exported from Vivado 2022.2.

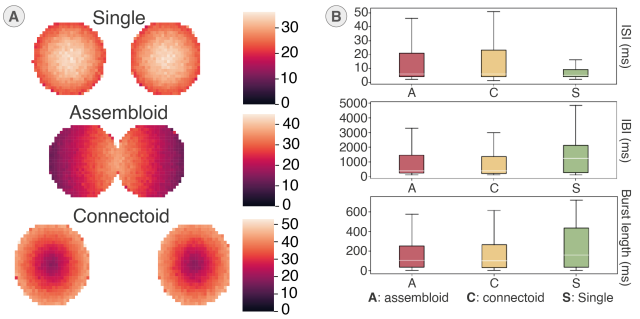

Fig. S7 Characterisation of the organoid modeling on BiœmuS. **(A)** Heatmap of the number of connection per neuron for the three structure of organoid interconnections. Single corresponds to the synaptic connection inside both organoids. Assembloid corresponds to a higher synaptic connection at the interface between the organoids. Connectoid favors synaptic connection on the exterior ring of the organoid. Heatmap are based on an average of 40 random generation of synapses for a given XY mapping. **(B)** Inter-Spike Interval (ISI), Inter-Burst Interval (IBI) and burst length of of the three structures based on a 10 seconds raster plot.

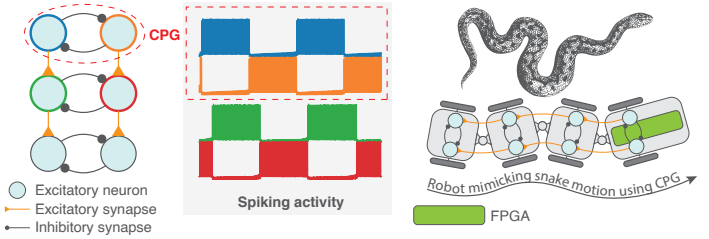

Fig. S8 Example of reduced version of BiœmuS applied to a smaller target for bioinspired application. Simple network connection reproducing Central Pattern Generator (CPG) network is implemented to reproduce snake motion on a robot. CPG are interconnected by excitatory synapses introducing delay. A robot featuring a motor for each neuron connected to the SNN is designed to enable motion upon spiking activity.

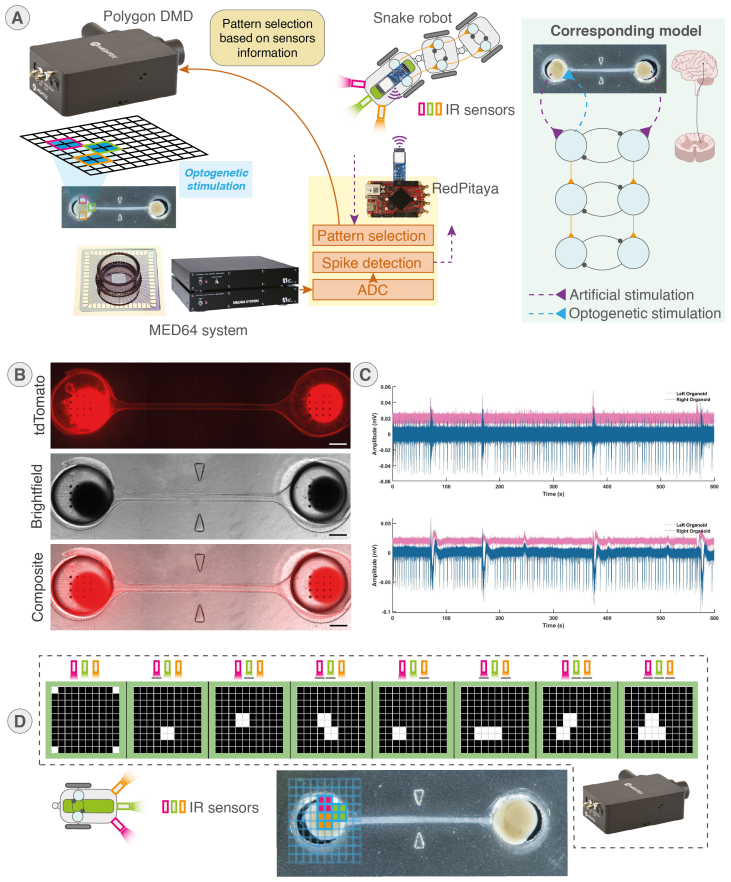

Fig. S9 Closed-loop biohybrid experiment integrating an embedded version of BiœmuS. **(A)** Closed loop interaction between biological connected cortical organoids and artificial CPG as a model of brain to spinal cord interaction. Spiking activity detected on the left and right organoids is forwarded through WiFi to the first neurons of the artificial CPG implemented on the robot shown from [2]. Three IR sensors located at the front of the the robot (purple,green and orange rectangles) trigger localized optogenetic stimulation on the left organoids using DMD based on the data transmitted by WiFi. **(B)** Connectoid organoids expression of ChR2 imaged at day 100. Infection and plating at day 60. Scale bar is 500µm. **(C)** Bandpass filtered (top) and raw (bottom) electrical activity of left and right organoids on day 92 recording on MEA. Bandpass filter has low-cut of 300 Hz and high-cut of 3kHz. **(D)** Sequence of DMD patterns selected based on infrared sensors information. The pattern were switched using the trigger in input of the DMD Polygon1000 that moves to next pattern upon reception of a pulse. The pattern was projected on the left organoid.

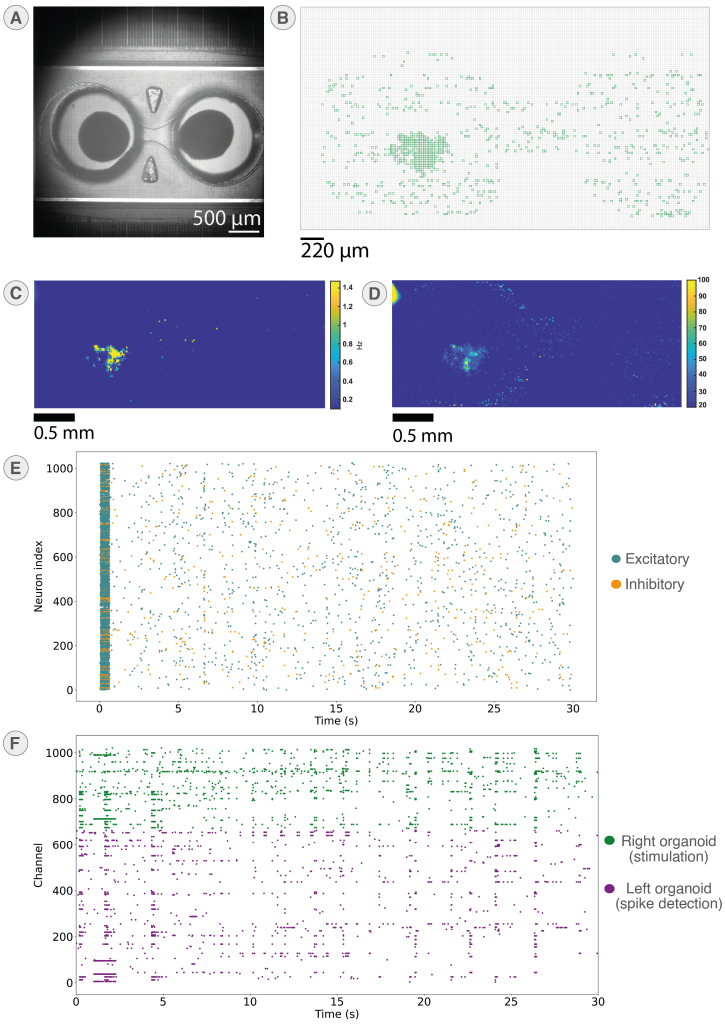

Fig. S10 Setup configuration for closed-loop stimulation on HD-MEA. **(A)** Connectoid organoids on MaxOne HD-MEA at d84 in device. **(B)** Electrode configuration of the HD-MEA chip for the experiments. Electrodes configuration is based on an activity scan carried out with MaxLab Live Software. **(C)** Firing rate map of the connectoid showing higher activity in the left organoid one day before the experiment. **(D)** Spike amplitude map of the connectoid one day before the experiment. **(E)** Spontaneous activity of the emulated single organoid emulated by BiœmuS. Green dots represents excitatory neurons (RS) and orange dots the inhibitory neurons (FS). Burst occurs only at initialization due to identical initial values for the parameters of the neurons. **(F)** Spontaneous activity of the connectoid on HD-MEA chip one day before the experiment. Green dots correspond to the channels recording from the right organoid that receives the stimulation. Purple dots correspond to the activity of the left organoid that triggers stimulation to BiœmuS based on spiking activity.

3 Supplementary Tables

Table S1 Tunable parameters from the Python scripts and data coding in the system.

Parameter	Coding	Unit
	Neurons	
g_{Na}	float32	S/cm^2
g_K	float32	S/cm^2
g_M	float32	S/cm^2
g_L	float32	S/cm^2
g_T	float32	S/cm^2
g_{Leak}	float32	S/cm^2
E_{Na}	float32	mV
E_K	float32	mV
E_{Ca}	float32	mV
E_{Leak}	float32	mV
v_{init}	float32	mV
μ_{noise}	sfixed18	1
θ_{noise}	sfixed18	1
σ_{noise}	sfixed18	1
I_{stim}	sfixed18	mA/cm^2
$area$	float32	cm^2
c_{mem}	float32	$\mu F/cm^2$
	Ionic channels[1]	
m_{Na}	float32	1
m_K	float32	1
m_M	float32	1
m_L	float32	1
m_T	float32	1
h_{Na}	float32	1
h_L	float32	1
h_T	float32	1
	Synapses	
w_{syn}	sfixed14	1
g_{AMPA}	sfixed18	nS
g_{NMDA}	sfixed18	nS
g_{GABA_a}	sfixed18	nS
g_{GABA_b}	sfixed18	nS
α_{AMPA}	ufixed17	$M^{-1}.s^{-1}$
α_{NMDA}	ufixed17	s^{-1}
α_{GABA_a}	ufixed17	$M^{-1}.s^{-1}$
β_{AMPA}	ufixed17	s^{-1}
β_{NMDA}	ufixed17	$M^{-1}.s^{-1}$
β_{GABA_a}	ufixed17	s^{-1}
$K1_{GABA_b}$	ufixed17	$M^{-1}.s^{-1}$
$K2_{GABA_b}$	ufixed17	s^{-1}
$K3_{GABA_b}$	ufixed17	s^{-1}
$K4_{GABA_b}$	ufixed17	s^{-1}
Kd_{GABA_b}	ufixed18	1
T_V[1]	ufixed17	M
B_V[1]	ufixed11	1

Max,min and step for float32 are not relevant since limited by model stability.

[1]Tunable through the equation stored in the ionic rate tables

Table S2 Parameters of the Hodgkin-Huxley model for the 4 preset types of neuron tunable from the Python scripts.

Parameter	FS	RS	IB	LTS	Unit
g_{Na}	0.05	0.05	0.05	0.05	S/cm^2
g_K	0.01	0.005	0.005	0.005	S/cm^2
g_M	0.0	7×10^{-5}	3×10^{-5}	3×10^{-5}	S/cm^2
g_L	0.0	0.0	1×10^{-4}	0.0	S/cm^2
g_T	0.0	0.0	0.0	4×10^{-4}	S/cm^2
g_{Leak}	0.00015	0.0001	1×10^{-5}	1×10^{-5}	S/cm^2
E_{Na}	50.0	50.0	50.0	50.0	mV
E_K	-100.0	-100.0	-90.0	-100.0	mV
E_{Ca}	0.0	0.0	120.0	120.0	mV
E_{Leak}	-70.0	-70.0	-70.0	-75.0	mV
v_{init}	-70.0	-70.0	-70.0	-75.0	mV
μ_{noise}	0.048	0.042	0.042	0.042	1
θ_{noise}	8.0	8.0	8.0	8.0	1
σ_{noise}	0.11	0.09	0.09	0.09	1
I_{stim}	0.03	0.03	0.03	0.03	mA/cm^2
$area$	$(67 \times 10^{-4})^2$	$(96 \times 10^{-4})^2$	$(96 \times 10^{-4})^2$	$(96 \times 10^{-4})^2$	cm^2
c_{mem}	1.0	1.0	1.0	1.0	$\mu F/cm^2$

Table S3 Configuration parameters of the C++ application setup from the JSON configuration file.

Key	Description
fpath_hwconfig	Path to hardware configuration file
emulation_time_s	Emulation time from 1 to 2^{32} s by 1 s
sel_nrn_vmem_dac	List of 8 neuron waveforms to select on DAC
sel_nrn_vmem_dma	List of 16 neuron waveforms to select on DMA
save_local_spikes	Enable/disable spike local saving
save_local_vmem	Enable/disable waveform local saving
save_path	Path to local saving file
en_zmq_spikes	Enable/disable ZeroMQ spike sending
en_zmq_vmem	Enable/disable ZeroMQ waveforms sending
en_zmq_stim	Enable/disable ZeroMQ external stimulation
en_WiFi_spikes	Enable/disable WiFi spike sending
ip_zmq_spikes	IP address ZeroMQ spike sending
ip_zmq_vmem	IP address ZeroMQ waveform sending
ip_zmq_stim	IP address ZeroMQ external stimulation
nb_tstamp_per_spk_transfer	Time stamps to wait for spike collection (x1 ms)
nb_tstep_per_vmem_transfer	Time steps to wait for waveform collection (x31.25 µs)
en_stim	Enable/Disable simulation step
stim_delay_ms	Stimulation step delay in ms
stim_duration_ms	Stimulation step duration in ms

4 Supplementary Equations

Pre-computed rate table for ionic channel state using forward Euler method

$$r_1(V) = 1 - \frac{dt}{tau_x(V)} = 1 - dt \times (\alpha_x(V) + \beta_x(V)) \tag{S1}$$

$$r_2(V) = \frac{dt \times x_\infty(V)}{tau_x(V)} = dt \times \alpha_x(V) \tag{S2}$$

where r_1 and r_2 are the pre-computed rate table for ionic channel states decoded from the membrane voltage, dt the time step in ms, $tau_x(V)$, x_∞, α_x and β_x the equation of the ionic channel state depending on the formalism used.

5 Supplementary Video

Movie S1

MaxOne chip stimulation based on burst detection from spiking activity of BicœmuS. Top left window is MaxLab Live Software visualisation of the chip, top right the received spiking activity from BicœmuS. Bottom left is the remote control of BicœmuS *via* terminal SHH and bottom right the debug information of the application executing both the burst detection, spike window and the stimulation sending.

References

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