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# 1 **A neuromorphic prosthesis to restore communication in neuronal** 2 **networks**

3 Stefano Buccelli<sup>1,2,3#</sup>, Yannick Bornat<sup>4#</sup>, Ilaria Colombi<sup>2,3\$</sup>, Matthieu Ambroise<sup>4\$</sup>, Laura  
4 Martines<sup>2,3\$</sup>, Valentina Pasquale<sup>3</sup>, Marta Bisio<sup>3,5</sup>, Jacopo Tessadori<sup>3,6</sup>, Przemysław Nowak<sup>2,7</sup>,  
5 Filippo Grassia<sup>4,8</sup>, Alberto Aversa<sup>1,2,3</sup>, Mariateresa Tedesco<sup>2</sup>, Paolo Bonifazi<sup>9,10,11</sup>, Francesco  
6 Difato<sup>3</sup>, Paolo Massobrio<sup>2</sup>, Timothée Levi<sup>4,12\*</sup> & Michela Chiappalone<sup>1,3\*^</sup>

7 <sup>#</sup>These authors contributed equally;

8 <sup>\$</sup>These authors contributed equally;

9 <sup>\*</sup>Corresponding authors

10

## 11 **Affiliations**

12 1 Rehab Technologies IIT-INAIL Lab, Istituto Italiano di Tecnologia, Via Morego 30, 16163  
13 Genova, Italy;

14 2 Department of Informatics, Bioengineering, Robotics, System Engineering (DIBRIS),  
15 University of Genova, Via all'Opera Pia 13, 16145, Genova, Italy;

16 3 Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Via  
17 Morego 30, 16163, Genova, Italy;

18 4 Laboratoire de l'Intégration du Matériau au Système (IMS), University of Bordeaux,  
19 Bordeaux INP, CNRS UMR 5218, 351 Cours de la Libération, 33405 Talence Cedex, France;

20 5 Department of Neurosciences, University of Padova, Via Nicolò Giustiniani 5, 35128,  
21 Padova, Italy;

22 6 Department of Advanced Robotics, Istituto Italiano di Tecnologia, Via Morego 30, 16163  
23 Genova, Italy;

24 7 Institute of Information Technology, Lodz University of Technology, ul. Wolczanska 215,  
25 90-924, Lodz, Poland;

26 8 University of Picardie Jules Verne, Laboratory of Innovative Technologies (LTI, EA 3899),  
27 Avenue des Facultés, Le Bailly, 80025 Amiens, France;  
28 9 School of Physics and Astronomy, Tel Aviv University, 69978 Tel Aviv, Israel;  
29 10 Computational Neuroimaging Laboratory, Biocruces Health Research Institute, Hospital  
30 Universitario Cruces, 48903, Baracaldo, Vizcaya, Spain  
31 11 Ikerbasque: The Basque Foundation for Science, 48013 Bilbao, Bizkaia, Spain  
32 12 LIMMS CNRS-IIS, The University of Tokyo, 153-8505 Tokyo, Japan

33

34 ***^Lead Contact***

35 ***Dr Michela Chiappalone, PhD***

36 Rehab Technologies IIT-INAIL Lab, Istituto Italiano Di Tecnologia, Via Morego 30, 16163

37 Genova

38 Tel: +39 010 71781743

39 E-mail: [michela.chiappalone@iit.it](mailto:michela.chiappalone@iit.it)

40

41 **\*Corresponding authors**

42 ***Dr Michela Chiappalone, PhD***

43 Rehab Technologies IIT-INAIL Lab, Istituto Italiano Di Tecnologia, Via Morego 30, 16163

44 Genova

45 Tel: +39 010 71781743

46 E-mail: [michela.chiappalone@iit.it](mailto:michela.chiappalone@iit.it)

47

48 ***Associate Prof Timothée Levi, PhD***

49 Laboratoire de l'Intégration du Matériau au Système (IMS), University of Bordeaux,

50 Bordeaux INP, CNRS UMR 5218, 351 Cours de la Libération, 33405 Talence Cedex

51 Tel: +33 54000 3118

52 E-mail: [timothee.levi@u-bordeaux.fr](mailto:timothee.levi@u-bordeaux.fr)

53 LIMMS, CNRS–Institute of Industrial Science, UMI 2820, The University of Tokyo,

54 4-6-1 Komaba, Meguro-ku, Tokyo, 153-8505, Japan

55 Tel: +81 3 5452 6721

56 E-mail: [levi@iis.u-tokyo.ac.jp](mailto:levi@iis.u-tokyo.ac.jp)

57

58

59 **Summary**

60 Recent advances in bioelectronics and neural engineering allowed the development of brain machine  
61 interfaces and neuroprostheses, capable of facilitating or recovering functionality in people with  
62 neurological disability. To realize energy-efficient and real-time capable devices, neuromorphic  
63 computing systems are envisaged as the core of next-generation systems for brain repair. We  
64 demonstrate here a real-time hardware neuromorphic prosthesis to restore bidirectional interactions  
65 between two neuronal populations, even when one is damaged or missing. We used *in vitro* modular  
66 cell cultures to mimic the mutual interaction between neuronal assemblies and created a focal lesion  
67 to functionally disconnect the two populations. Then, we employed our neuromorphic prosthesis for  
68 *bidirectional bridging* to artificially reconnect two disconnected neuronal modules, and for *hybrid*  
69 *bidirectional bridging* to replace the activity of one module with a real-time hardware neuromorphic  
70 Spiking Neural Network. Our neuroprosthetic system opens avenues for the exploitation of  
71 neuromorphic-based devices in bioelectrical therapeutics for healthcare.

72

73 **Keywords:**

74 *In vitro* neuronal networks; micro-electrode arrays; real-time signal processing; closed-loop;  
75 neurobiohybrid; brain repair.

76

## 77 **Introduction**

78 One of the greatest challenges of modern neuroscience is to find reliable and sustainable  
79 treatments for the disabling effects caused by many chronic and incurable brain conditions.  
80 With the greatest impact carried by stroke (Feigin et al. 2017) and traumatic brain injury  
81 (Maas et al. 2017), brain disorders are among the leading causes of disabilities worldwide.  
82 Due to recent advances in bioelectronics and in neural and neuromorphic engineering, direct  
83 interfacing of artificial circuits with large neuronal networks is possible to develop novel  
84 ‘neurobiohybrid’ systems (such as neuroprostheses (Vassanelli and Mahmud 2016)), which  
85 are envisaged as potentially interesting clinical applications for brain lesions (Broccard et al.  
86 2017). In this paper, we introduce an innovative bioelectronic system acting as a  
87 neuroprosthesis which, thanks to a neuromorphic real-time hardware interface, can re-  
88 establish the communication between two disconnected neuronal populations.

89         Neural interfaces are promising solutions for brain repair (Soekadar et al. 2015).  
90 Modern neural interfaces are mainly designed to restore lost motor functions in only one  
91 direction, i.e., from the brain to the body (Abdulkader et al. 2015) or from the body to the  
92 brain (Flesher et al. 2016). Additionally, recent neuroprosthetic developments have shown the  
93 enormous potential of neural interfaces to aid and accelerate functional recovery (Bouton et  
94 al. 2016; Rosin et al. 2011). However, a major obstacle in developing novel neuroprostheses  
95 for bidirectional communication with and within the brain is the complex nature of  
96 interactions among different brain areas, which in turn presents a challenge for the  
97 development of appropriate stimulation protocols as well as for testing such devices using *in*  
98 *vivo* models (Kohler et al. 2017).

99         Despite very recent technological progress (Jun et al. 2017; Steinmetz et al. 2018), *in*  
100 *vivo* models still have two main bottlenecks. The first bottleneck is the technical challenge to  
101 faithfully reproduce specific/focal network lesions (mainly due to their complexity) that the

102 neuroprosthesis aims to treat, whereas the second is the difficulty in disentangling the actual  
103 effect of the adopted electrical therapy from the complex activity of a brain constantly  
104 processing sensory inputs and producing behaviour. Therefore, since *in vivo* models exhibit  
105 inherent complexity and low controllability, using *in vitro* reduced neuronal systems to model  
106 precise and reproducible neuronal network lesions and test neuroprosthetic devices for their  
107 treatment may be advantageous. This approach is also justified by a growing recognition that  
108 *in vitro* testing of both research and medical devices can be more effective in terms of cost,  
109 time consumption and ethical issues and much more reliable than *in vivo* testing (Myers et al.  
110 2017).

111         In this work, we bidirectionally interfaced in real-time a neuroprosthetic system with  
112 an *in vitro* culture constituted by interconnected ‘neuronal assemblies’ (Hebb 1949).  
113 Therefore, our first objective was to create a simplified yet plausible *in vitro* model of a focal  
114 brain lesion by using bimodular cultures grown onto micro-electrode arrays (MEAs) (Bisio et  
115 al. 2014; Bonifazi et al. 2013; Shein-Idelson et al. 2011) with reciprocal connections cut by a  
116 custom-made laser setup (Difato et al. 2011) to mimic the pathological effect of a traumatic  
117 brain injury (Hayes et al. 2016). We created a neurobiohybrid system connecting the  
118 biological element (the bimodular culture) following the lesion with a neuroprosthetic  
119 prototype. Our hardware neuroprosthesis could perform low-power computations in hard  
120 real-time (Pirog et al. 2018), collecting the inputs coming from neural recordings, processing  
121 those signals and generating suitable electrical stimulation triggers as an output. With this  
122 experimental setup, we tested two specific applications, namely, *bidirectional bridging* (BB)  
123 to artificially reconnect two disconnected neuronal modules and *hybrid bidirectional bridging*  
124 in which a real-time spiking neural network (SNN) replaced the activity of one of the two  
125 modules in real-time while implementing bidirectional connectivity with the remaining  
126 neuronal module.

127           The motivation of our research is to provide a new technological instrument as a  
128 novel form of neuroprosthesis aimed at treating disabling brain pathologies. The hardware  
129 choice (field-programmable gate array, FPGA) maximise the real-time performances of the  
130 system and allows for a faster development of a future implantable bioelectronic device for  
131 biomedical applications. The adoption of bidirectional communication allows the  
132 development of a generalized non-specific approach that is applicable to the central nervous  
133 system (CNS) or peripheral nervous system. In particular, prostheses for the CNS should  
134 restore the communication between two or more neuronal assemblies whose functional and  
135 anatomical path could be distributed and sparse and not necessarily known *a priori*.

136           Indeed, our idea to develop a generalized approach comes from the future perspective  
137 of creating a cerebral neuroprosthesis for direct implantation in the brain that could be used  
138 by patients affected by stroke or brain injury. Our proof-of-principle results are the first for a  
139 next-generation neurobiohybrid system to restore brain functions (Broccard et al. 2017;  
140 Vassanelli and Mahmud 2016).

141



142 **Results**

143

144 *Neuroprosthetic architecture*

145 To create bimodular *in vitro* systems, we developed PDMS masks with two connecting  
146 compartments that constrained the growth of neuronal cells in two precise areas over 60-  
147 electrode MEAs (Figure S1). The obtained bimodular neuronal culture constitutes the  
148 biological neuronal network (BNN) of our system (Figure 1A). The signal from the BNN was  
149 amplified by a commercial system and acquired by a custom-developed FPGA-based  
150 neuromorphic board (Figure S2) previously configured by a custom-made MATLAB code  
151 (MathWorks, Natick, MA, USA) running on a general purpose personal computer. The  
152 neuromorphic board triggered a commercial stimulator to close the loop with the BNN. The  
153 general protocol designed for this study involved three steps. First, spontaneous activity in  
154 both neuronal modules was recorded ('pre-lesion condition'). Then, laser ablation of the  
155 biological connections between the two modules was performed (see Transparent Methods),  
156 followed by recording of spontaneous activity in both modules ('post-lesion condition') to  
157 assess the viability of the networks. Finally, we tested our neuroprosthetic device using two  
158 experimental frameworks. In the first case, we applied a reconnection strategy using a  
159 bidirectional activity-dependent stimulation ('bidirectional bridging', BB), whereas in the  
160 second case, we interfaced a hardware-implemented biomimetic SNN with one of the two  
161 neuronal modules ('hybrid bidirectional bridging', HBB) to simulate a 'replacement' strategy  
162 that utilizes the bidirectional interaction between the biological system and its artificial  
163 counterpart (Figure 1B). The sequence of algorithms (e.g., spike detection, network burst  
164 detection) implemented on the board to realize both experimental approaches (BB or HBB) is  
165 schematically depicted in Figure 1C.

166

167

<FIGURE 1 ABOUT HERE>

168

169 ***Control experiments***

170 To evaluate the stability and effect of the focal lesion on bimodular BNN activity, we first  
171 performed two sets of control experiments. In the first set defined as ‘experiments with no  
172 lesion’ (Figure 2A1), we recorded 4 consecutive hours of spontaneous activity (S1-S4, n=9  
173 cultures). In the second set defined as ‘experiments with a lesion’ (Figure 2A2), we recorded  
174 one hour of spontaneous activity (S1), followed by laser ablation of the connections between  
175 the two modules, which usually took less than 20 minutes. Next, we recorded 3 hours of  
176 spontaneous activity post lesion (SPL1-SPL3) to quantify the effects of laser ablation (n=4  
177 cultures). As depicted in the raster plot of one representative experiment (Figure 2B1),  
178 bimodular neuronal networks exhibited spontaneous, synchronized, multi-unit activity  
179 composed of network-wide bursts (NBs) spreading over the two modules. Following laser  
180 ablation, the propagation between compartments was disrupted, as shown in Figure 2B2.

181 With no lesion, the percentage of active channels with respect to the first hour of  
182 recording (S1) was higher than 98% and was maintained for the entire duration of the  
183 experiment (Figure 2D1, light blue bars). The mean firing rate (MFR) was stable for all  
184 control experiments with no lesion (from S1 to S4, Figure S3 A). Alternatively, control  
185 experiments with lesions showed a reduced number of active channels (close to 73%) during  
186 the first hour after ablation (SPL1). During the following two hours (SPL2-SPL3), this value  
187 increased and reached 93% at the end of the recording (Figure 2C1, dark grey bars). No  
188 significant differences were found. The MFR was quite stable for all control experiments  
189 with lesions except between S1 and SPL1 (Figure S4 A). The activity level with respect to  
190 the S1 phase, expressed by the MFR ratio with respect to S1, was stable during the control  
191 experiments without lesions (Figure 2D2, light blue bars). The lesion produced a clear

192 decrease in activity in most cultures, especially during the first two hours (SPL1-SPL2,  
193 Figure 2D2). We found a significant difference between the two experimental sets during the  
194 first two hours after S1 but not during the last hour. This result suggests that two hours after a  
195 lesion, almost complete spontaneous recovery occurred in terms of the firing rate for the two  
196 neuronal modules.

197 To evaluate changes in the synchronicity between the two modules, we performed  
198 cross-correlation (CC) analysis between the collapsed spike trains of each module. The shape  
199 of the CC function was stable throughout the entire recordings in experiments without  
200 lesions, as reported in Figure 2C1 for a representative experiment. After a lesion, the CC  
201 function collapsed to zero and did not recover during the experiment (Figure 2C2:  
202 representative experiment). To quantify this difference, we integrated the CC function in a  
203 range of  $\pm 500$  ms to obtain the CC area. We did not find any significant change in the CC  
204 area values for all experiments with no lesion (Figure S3B). By contrast, the CC area values  
205 showed a marked decrease following the lesion (SPL1). This decrease was due to the lack of  
206 anatomical connections between the compartments and did not recover by itself (Figure  
207 S4B). Comparing the CC area ratio between later phases and S1 resulted in a significant  
208 difference between matching periods in 'lesion' and 'no lesion' experiments (Figure 2E1).  
209 We also computed the correlation coefficient (i.e. Pearson Correlation, PC) among all the  
210 active channels both intra module and inter module (Figure 2E2). The intra-module PC was  
211 constant across experimental phases for controls with no lesion (light blue bars). On the other  
212 hand, for controls with lesion (dark grey bars) there was a drop in the intra-module PC,  
213 related to the reduced firing rate following the lesion, but no statistical difference was found  
214 (Figure 2E2, left panel). The inter-module CC was stable for controls with no lesion. On the  
215 other hand, following the lesion the inter-module PC collapsed and never recovered by itself  
216 (Figure 2E2, right panel) as already demonstrated with the previous analysis.

217 The network bursting rate (NBR) was stable during all experiments with no lesions  
218 (Figure S3C). For the experiments with lesions, this parameter was less stable but with no  
219 significant differences between phases (Figure S4C). When comparing the two experimental  
220 protocols with the NBR ratio with respect to S1, we found significant differences during the  
221 first and the second hour post lesion (Figure 2F1). The mean probability to have NBs  
222 composed of spikes belonging to a single module (i.e. Prob smNB, see Transparent Methods)  
223 was close to 0.2 in the experiments without lesions (Figure S3D), meaning that the majority  
224 of NBs in an intact bimodular network involved both modules. Alternatively, following the  
225 lesion, the probability became close to 1 (Figure S4D), meaning a total loss of functional  
226 communication between the two compartments. Using the Prob smNB ratio with respect to  
227 S1 (Figure 2F2), we found significant differences between the two experimental groups  
228 during all phases post lesion (Mann-Whitney test;  $p < 0.05$ ). Thus, for the no lesion  
229 experiment, the Prob smNB remained very similar to the initial values, while for the lesion  
230 experiments, it changed abruptly due to the lesion. This result further confirmed that the  
231 lesion was effective in functionally disconnecting the two modules.

232

233 < FIGURE 2 ABOUT HERE >

234

### 235 ***Experiment 1: Bidirectional Bridging (BB)***

236 The goal of this experiment is to restore communication between two neuronal assemblies  
237 after lesion-induced separation. To achieve this goal, we designed and implemented a  
238 stimulation reactive paradigm inspired by the ‘activity-dependent stimulation’ (ADS)  
239 described in (Guggenmos et al. 2013) in our neuromorphic board. In contrast to the control  
240 experiments, the general protocol (Figure 3A) included 20 minute recordings of spontaneous  
241 activity before the lesion (S1). Upon the lesion execution, we waited for two hours to reach

242 stable activity in both modules, as shown by the results of control experiments (see Figure 2).  
243 Then, we recorded 20 minutes of spontaneous activity (SPL3). The raster plot of a  
244 representative experiment is reported in Figure 3b. Before the lesion (S1), the bursting  
245 activity involved both modules (Figure 3B1), whereas after the lesion (SPL3), the activity  
246 was characterized by single-module NBs (Figure 3B2). To choose the best parameters  
247 (threshold and window time, see Transparent Methods) that allowed us to reliably detect NBs  
248 in both modules, we performed offline NB detection. After the FPGA was updated with these  
249 parameters, a 20 minute session of BB was conducted. (see, Figure 1B and C for the  
250 description of the BB protocol). The BB approach implemented a reactive paradigm: every  
251 time an NB was detected in one module, a stimulation pulse was delivered to an electrode in  
252 the other module (see Transparent Methods) in both directions. During the BB phase, the  
253 bursting activity involved both modules similar to the intact condition due to the bidirectional  
254 stimulation pulses (Figure 3B3, blue and red lines represent electrical stimulation pulses  
255 delivered from module 1 to module 2 and vice versa). The last phase of the protocol involved  
256 20 additional minutes of spontaneous activity (SPL4), which showed the same activity as  
257 SPL3 (Figure 3B4). We did not observe significant changes in spiking activity (i.e., MFR)  
258 throughout the recordings (Figure 3c).

259         Next, we evaluated the effect of this configuration in terms of CC (Figure 3d1 and 2).  
260 During spontaneous activity before the lesion (S1), the CC peak was high and stable due to  
261 the functional and anatomical connections between the two modules, which was also reported  
262 for the control experiments. After the lesion (SPL3), there was a decrease in CC that was not  
263 expected to recover without external intervention, as we demonstrated before (see, Figure  
264 2D). The bidirectional stimulation at least partially recovered the CC area and consequently  
265 the communication between modules (Figure 3D2), as demonstrated by statistical analysis.  
266 Regarding the number of NBs, we did not find any significant difference between the

267 experimental phases (Figure 3E1). However, the probability of isolated NBs was not uniform;  
268 it reached the maximum value after the lesion, as we previously observed in the control  
269 experiments with lesions (see, Figure 2D2). During bidirectional stimulation, these values  
270 became closer to the spontaneous recording (Figure 3E2), meaning that NBs mainly involved  
271 both modules. This finding further confirmed that the BB protocol could reconnect two  
272 disconnected modules through a real-time ADS acting in both directions (from module 1 to  
273 module 2 and from module 2 to module 1).

274

275 <FIGURE 3 ABOUT HERE>

276

### 277 ***Experiment 2: hybrid bidirectional bridging (HBB)***

278 With an injury causing damage to an entire neuronal subnetwork, a reconnection strategy  
279 such as the BB illustrated above would not be feasible. For this reason, we developed a  
280 second reconnection strategy based on the use of a hardware SNN that can interact in real-  
281 time with its biological counterpart, HBB (see Figure 1). We created a set of SNNs (i.e., SNN  
282 library) by tuning the mean value of the synaptic weight distributions of our models to cover  
283 the variability of the BNNs (i.e., BNN library, Figure 4A). The biomimetic SNN (see Figure  
284 S5), working in hardware real-time to allow bidirectional communication with living  
285 neurons, was modelled as a network of 100 Izhikevich (IZH) neurons (Izhikevich 2003), with  
286 80 excitatory and 20 inhibitory neurons (see Transparent Methods), according to the  
287 biological composition of dissociated cultures (Bonifazi et al. 2005; Hayashi et al. 2003).  
288 Synaptic noise (Grassia et al. 2016), inhibitory and excitatory synapses (Izhikevich 2004),  
289 short-term plasticity (Izhikevich and Edelman 2008) and axonal delays were included in the  
290 model to recreate the network dynamics (see Transparent Methods and Figure S6 A1 and  
291 A2). Regarding the connectivity rules, we set the outdegree (i.e., the number of post-synaptic

292 neurons) to 25 for all neurons in the network, while the indegree (i.e., the number of pre-  
293 synaptic neurons) followed a normal distribution with a mean value of 25 and a standard  
294 deviation of 4.3 (Figure S6, R-Square=0.806). The goal of creating an SNN library was to  
295 cover a wide range of NBRs because NB was chosen as the triggering event for our  
296 reconnection paradigm, as explained above. To this end, we tuned only the mean value of the  
297 normal distribution of synaptic weights (the standard deviation was kept constant at the value  
298 of 0.3). By increasing or decreasing the mean synaptic weights, we tuned the NBR. For  
299 excitatory synapses, the mean value ranged from 0.99 to 1.34 (Figure 4C left), while that for  
300 inhibitory synapses ranged from -2.02 to -1.02 (Figure 4C right and Table S1). As previously  
301 stated, our goal was to cover the NBR variability and not the MFR. The MFR variability in  
302 our BNN library was higher than that obtained with our SNN library (Figure 4E and F1).  
303 Nevertheless, the BNN variability in terms of NBR was completely covered by our SNN  
304 library, which also contains networks with a much higher NBR than that in the BNN library  
305 (Figure 4F2).

306  
307 <FIGURE 4 ABOUT HERE>  
308

309 The general HBB protocol (Figure 5A) is similar to the BB protocol. The HBB  
310 procedure included a 20 minute recording of spontaneous activity before the lesion. This  
311 recording was used to quantify activity in terms of the NB rate of the network. This feature  
312 was used to choose one SNN from the SNN library that had an NB rate closer to its biological  
313 counterpart. We waited two hours after the lesion to allow activity in both modules to  
314 stabilize, as shown by the results of control experiments (e.g., Figure 2). Then, we recorded  
315 20 minutes of spontaneous activity. After setting FPGA detection parameters, we performed a  
316 20 minute HBB session (see, Figure 1B and C for the description of the HBB protocol). As  
317 anticipated, the HBB approach also implemented an ADS paradigm; every time an NB was

318 detected on the 'surviving' module (i.e., when one of the two modules was completely  
319 damaged), a stimulation pulse was delivered to the SNN. The board implemented the  
320 corresponding paradigm in the opposite direction. Detection of NBs occurred in the SNN,  
321 while stimulation was delivered to the 'surviving' module, thus avoiding the imposition of  
322 any predefined unidirectional communication. Next, we recorded 20 additional minutes of  
323 spontaneous activity.

324 We did not observe significant changes in terms of spiking activity (i.e., MFR)  
325 throughout the recordings (Figure 5C). Then, we evaluated the effect of this configuration in  
326 terms of CC (Figure 5D1 and 2). During spontaneous activity before the lesion (S1), the CC  
327 peak was high and stable due to the functional and anatomical connections between the two  
328 modules, which was also reported for the control experiments. As expected, with no external  
329 intervention, CC decreased sharply after the lesion (SPL3), as we previously observed. One  
330 of the two modules was damaged, while the correlation was evaluated between the SNN and  
331 the surviving module during the HBB phase. The bidirectional stimulation created a relevant  
332 correlation area between SNN and the surviving module, as demonstrated by statistical  
333 analysis. Regarding the number of NBs, we did not find a significant difference between the  
334 S1 and HBB phases (Figure 5E1). However, the probability of isolated NBs was not uniform;  
335 it reached the maximum value after the lesion, as we previously observed in the control  
336 experiments with lesions (see, Figure 3E2). During the hybrid bidirectional stimulation, these  
337 values became closer to the spontaneous recording (Figure 5E2), meaning that NBs mainly  
338 involved both modules. This finding further confirmed that the HBB protocol created a  
339 hybrid system with the surviving biological module through real-time ADS acting in both  
340 directions (from BNN to SNN and from SNN to the BNN).

341

342 <FIGURE 5 ABOUT HERE>



## 343 **Discussion**

344 We presented an innovative neuromorphic prosthesis based on a FPGA board and  
345 demonstrated two successful reconnection paradigms for a lesion interrupting the  
346 communication between two neuronal populations *in vitro*.

347 According to previous reports, *in vitro* systems constitute a successful experimental  
348 model of neuronal dynamics (Javier et al. 2013; Johnson et al. 2010), thus providing an  
349 excellent test bed for adaptive closed-loop neural interfaces (Potter 2010). Starting from our  
350 recently developed methodology (Kanner et al. 2015), we created custom bimodular cultures  
351 with the goal of reproducing two interacting neuronal populations, thus mimicking the  
352 intrinsic modularity of the brain (Bonifazi et al. 2013). Our bimodular cultures were highly  
353 temporally stable in terms of firing properties at the whole network level as the activity  
354 between the two populations remained highly correlated for the entire duration of the  
355 recording. A lesion produced via laser ablation was employed to physically cut the  
356 connections between the two modules. This methodology was proven to be safe because it  
357 produced localized damage by selectively ablating subcellular compartments without  
358 damaging adjacent structures (Difato et al. 2011; Habibey et al. 2015; Soloperto et al. 2016).  
359 We assume that such a focal damage, allowing to specifically cut few connections among  
360 those available in the network, together with possible intrinsic compensatory mechanisms of  
361 synaptic scaling (le Feber et al. 2017; Turrigiano 2008), were responsible of the spontaneous  
362 recovery of the firing rate on a timescale of two-three hours. This demonstrates the  
363 effectiveness of our technique in preserving the functionality of the two modules while  
364 decoupling their activity, as proven by the loss of correlation of bursting behaviour.

365 Two different applications of our neuroprosthesis, BB and HBB, were tested. Our  
366 neuromorphic prosthesis, independently of the stimulation paradigm, works according to a  
367 closed-loop reactive policy as follows: each time a condition is met (i.e., an ‘event’ is

368 detected), a stimulus is delivered. The hardware architecture was designed to be flexible  
369 enough to allow the implementation of different experimental paradigms and the definition of  
370 different triggering events. In our study, we chose ‘NBs’ as trigger events (see Transparent  
371 Methods). The choice to deliver a stimulation depending on a network-wide event has two  
372 main advantages as follows: first, NB frequency is low enough to avoid inducing plasticity  
373 phenomena by electrical stimulation in our cultures (Wagenaar et al. 2006), which could  
374 confound the final results and effectiveness of neuroprosthetic reconnection. The second  
375 point is anticipation of the following major issue that will emerge during *in vivo* recordings:  
376 monitoring single neurons presents problems at both theoretical (Guggenmos et al. 2013) and  
377 practical levels. Namely, how much information on complex functions can be obtained by  
378 single-neuron observation remains unclear (Luczak et al. 2015; Panzeri et al. 2017), while  
379 tracking the activity of the same neuron for extended periods of time is problematic (Kozai et  
380 al. 2015). Taking multiple input sources into account was also used in the work of Berger  
381 (Berger et al. 2012), but they employed a neuroprosthetic strategy different from ours. In  
382 particular, these authors used a generalized linear model to predict the CA1 activity from  
383 spikes in CA3 of the hippocampal circuit. Our system is considered more flexible and  
384 adaptable to networks with different connectivity, not just feedforward similar to that in the  
385 hippocampus. Moreover, we were interested in mimicking the overall spiking activity of the  
386 network and not mapping an input-output transformation only.

387         Another important novelty of our system regards directionality. To our best  
388 knowledge, this neuroprosthetic system is the first to implement a truly bidirectional  
389 interaction with a SNN through a hard real-time interface. We recorded activity from the first  
390 module (via multiple sources); when a criterion was met, the device stimulated the second  
391 module (this is how a ‘typical’ closed-loop in neuroscience works, for a review see  
392 (Greenwald et al. 2016)). The novelty is simultaneously monitoring multiple sources from

393 another module and delivering the stimulation when the triggering event is detected. To date  
394 and as far as we are aware of, only Jung and colleagues (Jung et al. 2001) have performed a  
395 bidirectional interface to a neuromorphic device, but their models were not precise at the  
396 spike level (modelling neuron populations) and they used non configurable analogue  
397 electronics, which resulted in an experiment-specific setup. The other neuroprosthetic devices  
398 that have been proposed in the literature can implement a ‘unidirectional’ artificial link only  
399 from one area to another (or maybe the same) but not doubling it. Here, we are not imposing  
400 any preferred directionality to the communication; networks are self-organizing on the basis  
401 of their intrinsic natural relationship (we are not imposing who is driving whom). This  
402 approach has the main advantage of informing both brain regions (i.e., neuronal modules, in  
403 our case) that an event occurred in the other region, given that interaction in the brain is  
404 intrinsically bidirectional (Roelfsema and Holtmaat 2018). For example, in the sensorimotor  
405 system, sensory stimulation can help motor recovery (Cuppone et al. 2018), and motor  
406 learning can enhance sensory functions (Ostry et al. 2010; Takeuchi and Izumi 2013).  
407 Applications of our neuroprosthetic systems to conditions where the sensorimotor interaction  
408 is impaired would allow restoration of both communication channels, suggesting  
409 improvements in current rehabilitation therapies. Moreover, although tested on a bimodular  
410 system, the neuromorphic FPGA board can be easily upgraded to play the bridging role on an  
411 arbitrary number (within reason) of different neuronal circuits. A recent work (Forró et al.  
412 2018) developed directional networks of primary hippocampal neurons on MEA and  
413 compared the information flow of these networks with respect to bidirectional networks  
414 (similar to our bimodular preparations). They found that without physically imposing a  
415 unidirectional configuration, there is a continuous back and forth communication between  
416 nodes thus suggesting the importance of a bidirectional communication in a healthy network.

417           The second paradigm we tested was based on the use of a biomimetic SNN to  
418 ‘substitute’ a missing/damaged neuronal population. Currently, SNN applications span  
419 different fields, including computational neuroscience (Markram 2012; Melozzi et al. 2017),  
420 and very recently, they were used for sensory encoding in hand prosthesis for amputees  
421 (Osborn et al. 2018; Valle et al. 2018). SNNs can be simulated in software (Goodman and  
422 Brette 2009) and/or neuromorphic hardware (Thakur et al. 2018). As time and energy  
423 consumption are fundamental in neuroprosthetic applications for translational purposes, the  
424 use of hardware-based computing systems becomes mandatory.

425           In general, hybrid systems composed of *in vitro* BNNs coupled to SNNs are rare. In  
426 one approach, the SNN served as a self-organizing classifier of activity patterns exhibited by  
427 the BNN, with output of the SNN being subsequently used to control the behaviour of a robot  
428 (Pizzi et al. 2009). Other studies focused on the unidirectional or bidirectional influence of  
429 the two networks, investigating the dynamics of the interaction between the BNN and SNN in  
430 which the SNN played a role of an artificial counterpart of its biological original (Bruzzone et  
431 al. 2015; Chou et al. 2015). However, closed-loop effects in those hybrid networks were not  
432 thoroughly determined. In one of these studies, only unidirectional connectivity was  
433 considered with input from the SNN to the BNN, which was also simulated beforehand  
434 (Bruzzone et al. 2015). In this study, we established hybrid communication in the case of an  
435 entire neuronal population that needed substitution.

436           A study by Chou *et al.* (Chou et al. 2015) implemented a bidirectional interface  
437 between an SNN and a retinal slice obtained from an adult rat and recorded by an MEA. This  
438 system is quite interesting, but there is a 1 s delay between the BNN and SNN interactions.  
439 Therefore, this delay in Chou’s setup is 3 orders of magnitude larger than that in our work in  
440 which the sampling of biological activity is never interrupted, and the step size of the SNN is  
441 1 ms. The difference between the two systems is radical; bidirectional communication in real-

442 time allows actual clinical application, whereas delays in the range of seconds prevent (or at  
443 least seriously reduce) the possibility of meaningful control of a biological system.

444 A recent study, inspired by a previous work (Hogri et al. 2015), implemented a hybrid  
445 interaction (Xu et al. 2018) between the cerebellum of a rat and an SNN implemented on  
446 FPGA. Their model involved 10k leaky integrate and fire (LIF) neurons and did not integrate  
447 other biomimetic behaviours, such as axonal delay, short-term plasticity and synaptic noise,  
448 unlike the IZH neurons implemented in our system. Both the hard real-time processing and  
449 simplified neuronal model (which allow mimicking the richness of the electrophysiological  
450 patterns *in vivo*) are mandatory for reproducing the biological dynamics of living neural  
451 networks and for performing useful real-time hybrid experiments.

452 In this work, we demonstrated the possibility to design a neuromorphic all-hardware  
453 prosthesis capable of artificially reconnect two disconnected neuronal networks or artificially  
454 replacing one entire neuronal sub-network.

455

456 The use of a fully integrated hardware computing system allowed hard real-time  
457 performances and low power consumption, which are crucial for translational purposes  
458 related to therapeutic applications in humans (Kipke et al. 2008; Wang et al. 2010).

#### 459 ***Limitations of the study***

460 A limitation of the present work is that we deliberately chose to downsample both the  
461 number of biological neurons recorded through a low density MEA and the number of  
462 artificial neurons implemented on the FPGA. For the purpose of detecting network-wide  
463 activity, this oversimplification of biological complexity can be acceptable to test the  
464 functionality of the device and the feasibility of the approach, but if the goal is to functionally  
465 replace a biological network, a higher resolution would be preferable. It is worth underlying  
466 that this hardware implementation is not exploiting the full resources of the FPGA, thus in

467 follow-up studies, also thanks to the flexibility of our system, we foresee to scale up the  
468 number of neurons and synapses and to upgrade the computational algorithms to deal with  
469 more complex experimental designs. From a technological point of view, the current state of  
470 the art makes possible the use of devices with a large number (thousands) of recording  
471 electrodes (Berdondini et al. 2009; Frey et al. 2009). Such an improvement would also allow  
472 to have more information about functional connectivity of the biological network (Pastore et  
473 al. 2018) and thus developing more realistic, in terms of topology, artificial models. In this  
474 work, we arbitrarily modeled the network connectivity with a random adjacency matrix since  
475 the use of MEAs with 60 electrodes made impossible to correctly identify the topological  
476 properties (e.g., hubs, recurring connections, modules, etc.) of the network under  
477 investigation.

478 We are aware that the road is still long to target human applications. Despite this, we think  
479 that the extensive work performed represent an important milestone to start from. The next  
480 fundamental (and critical) step would be to test the neuromorphic prosthesis in vivo, for  
481 example on animal models affected by ischemic or traumatic lesions (Guggenmos et al.  
482 2013). Even if the adaptation to the new experimental set-ups will require time, we believe  
483 these are necessary steps to further push the translational potential of our system, which will  
484 be able to create real innovation in the clinical therapeutics.

485

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496 **Author contributions**

497 T. L., Y. B., P. M., P. B. and M. C. designed the study. Y. B. and T. L. designed and  
498 fabricated the hardware board. S. B., V. P., F. D., and M. C. designed the experiments. M. A.,  
499 P. M., P. N., F. G. and T. L. designed and worked on SNN. I. C., M. B., and M. T. prepared  
500 the bimodular cultures. S. B., L. M., A. A. and F. D. performed the experiments. S. B., L. M.,  
501 J. T., V. P., and M. C. designed the analyses. S. B. and L. M. performed the analyses. S. B., I.  
502 C., and V. P. performed the statistical analyses. S. B. and I. C. prepared the original figures.  
503 S. B., I. C., T. L., and M. C. wrote the manuscript. T. L., P. M., P. B. and M. C. acquired  
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505 version of the manuscript.

506

507 **Declaration of Interests**

508 The Authors report no competing interests.

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687

688

689 **Figure titles and legends**

690 **Figure 1. Interfacing a biological neural network and neuromorphic neuroprosthesis. A,**

691 Schematic representation of the main elements of the setup: cartoon of an MEA coupled with a BNN;  
692 picture of the amplification system; picture of the custom FPGA board; picture of the stimulus  
693 generator. Out of the loop, we used a PC to configure the board. **B**, Schematic representation of the  
694 different phases of two experimental approaches which share a pre-lesion, a lesion (performed  
695 through laser ablation, not shown) and a post lesion phase. The final experimental phase can be either  
696 bidirectional bridging (BB) or hybrid bidirectional bridging (HBB). **C**, Schematic of real-time data  
697 processing performed by the board: the first step is spike detection followed by network burst (NB)  
698 detection monitoring module 1. After NB detection, delivering stimulation to module 2 of the BNN  
699 (BB approach) or to the SNN is possible. In the second modality, there is also NB detection of the  
700 SNN, which can result in stimulation delivered to the BNN (HBB approach).

701

702 **Figure 2. A laser ablation-induced lesion can disconnect two neuronal modules. A1, Schematic of**

703 the first experimental protocol. Experiments with no lesion: we recorded four consecutive hours of  
704 spontaneous activity (S1-S4). **A2**, Schematic of the second experimental protocol. Experiments with  
705 lesion: we recorded one hour of spontaneous activity (S1) followed by laser ablation and three  
706 consecutive hours of spontaneous activity post lesion (SPL1-SPL3). The grey-shaded area indicates  
707 20 minutes of no recording due to the execution of the lesion. **B1**, A 20 s raster plot of the network  
708 bursting activity of one representative experiment during the S1 phase. **B2**, a 20 s raster plot of the  
709 network bursting activity of one representative experiment during SPL3. **C1**, Cross-correlation (CC)  
710 function for one representative experiment during the S1-S4 phases. The CC profiles between the  
711 spike trains of each module (light blue) in the four phases of the experiment were high and stable  
712 (lines shifted for the sake of clarity). Time axis [-500, +500] ms. **C2**, CC profile between the spike  
713 trains of each module for one representative experiment with lesion. Before the lesion (light blue  
714 profile), CC was high; following the lesion (dark grey), CC collapsed to zero (lines shifted for the  
715 sake of clarity). Time axis [-500, +500] ms. **D1** Percentage of active channels with respect to S1 for

716 the experiments with no lesions (light blue columns, n=9) and with lesions (n=4, dark grey columns).  
717 No significant difference was found using the Mann-Whitney test (S2 VS SPL1  $p = 0.2042$ ; S3 VS  
718 SPL2:  $p=0.31608$ ; S4 VS SPL3:  $p=0.70769$ ). **D2**, Mean firing rate (MFR) ratio with respect to S1 for  
719 experiments without (light blue bars) and with lesions (dark grey bars). No significant difference was  
720 found during the last hour using the Mann-Whitney test (S2 vs SPL1:  $p = 0.0028$ ; S3 vs SPL2:  $p =$   
721  $0.01119$ ; S4 vs SPL3:  $p = 0.10629$ ). **E1**, Comparison of the CC area ratio with respect to S1 for the  
722 experiments without (light blue bars) and with lesions (dark grey bars) (Mann-Whitney test; S2 vs  
723 SPL1:  $p = 0.0028$ ; S3 vs SPL2:  $p = 0.0028$ ; S4 vs SPL3:  $p = 0.0028$ ). **E2**, On the left, comparison of  
724 the intra-module correlation coefficient (i.e., Pearson Correlation, PC) ratio with respect to S1 for the  
725 experiments without (light blue bars) and with lesions (dark grey bars). No significant difference was  
726 found using the Mann-Whitney test (S2 VS SPL1  $p = 0.71049$ ; S3 VS SPL2:  $p= 0.14825$ ; S4 VS  
727 SPL3:  $p= 0.07552$ ). On the right, the same comparison regarding inter-module PC that showed clear  
728 differences between experiments without and with lesion (Mann-Whitney test; S2 vs SPL1:  $p$   
729  $=0.0028$ ; S3 vs SPL2:  $p = 0.0028$ ; S4 vs SPL3:  $p = 0.0028$ ). **F1**, Network burst rate (NBR) ratio with  
730 respect to S1 showing significant differences during the first and second hour after the lesion (Mann-  
731 Whitney test; S2 vs SPL1:  $p = 0.0028$ ; S3 vs SPL2:  $p = 0.01119$ ; S4 vs SPL3:  $p = 0.26014$ ). **F2**,  
732 Probability of single-module NB (Prob smNB). The ratio with respect to S1 shows stability for  
733 experiments without (light blue bars) and with lesions (dark grey bars) (Mann-Whitney test; S2 vs  
734 SPL1:  $p = 0.0028$ ; S3 vs SPL2:  $p = 0.0028$ ; S4 vs SPL3:  $p = 0.0028$ ).

735

736 **Figure 3. Bidirectional bridging is effective in reconnecting functionally and anatomically**  
737 **disconnected neuronal modules. A**, Schematic of the experimental protocol. We recorded 20  
738 minutes of spontaneous activity (S1) followed by laser ablation. The grey-shaded area indicates 20  
739 minutes of no recording during ablation. Dots represent two hours of no recording after the lesion to  
740 maintain a stable activity in both modules. Then, we recorded 20 minutes of SPL activity (SPL3)  
741 followed by 20 minutes of the bidirectional bridging (BB) protocol and another 20 minutes of  
742 spontaneous activity (SPL4). **B1-4**, The 20 s-long raster plots of representative experiments  
743 (respectively, from phases S1, SPL1, BB and SPL4). In **B3**, Blue and red lines represent electrical

744 stimulation pulses delivered from module 1 to module 2 and vice versa, respectively. **C**, MFR during  
745 the 4 experimental phases was stable (colour code as in panel a: S1: light blue dot; SPL3, SPL4: dark  
746 grey dots; BB: red dot). No significant difference was found (one-way RM ANOVA,  $p=0.469$ ,  $DF =$   
747  $3$ ,  $F = 0.872$ ) **D1**, CC function during the 4 experimental phases. Small arrows indicate the blanking  
748 period of 8 ms following each stimulation. Colour code the same as that in panel a. Note that during  
749 BB, the cross-correlation function (red) recovers even if not completely with respect to the initial  
750 profile (light blue), while it stays at zero during the spontaneous activity phases post lesion (SPL3 and  
751 SPL4, dark grey profiles). **D2**, CC area was highly reduced during the post-lesion phases. The CC  
752 area partially recovered during the BB protocol and collapsed again when stimulation was switched  
753 off (one-way repeated measures analysis of variance; degrees of freedom=3;  $F=101,832$ . S1 vs SPL3  
754  $p=5.67E-13$ ; S1 vs SPL4  $p=7.54E-13$ ; S1 vs BB  $p=1.60E-07$ ; BB vs SPL3  $p=1.77E-06$ ; BB vs SPL4  
755  $p=2.81E-06$ ; SPL4 vs SPL3  $p=1$ ). **E1**, NBR remained stable during the experiments. No significant  
756 difference was found (one-way repeated measures analysis of variance:  $p=0.501$ ,  $DF=3$ ;  $F=0.810$ ).  
757 **E2**, Probability of the single-module NB (Prob smNB) was close to one after the lesion. During the  
758 BB protocol, the probability was similar to the pre-lesion condition. (Friedman's repeated measures  
759 analysis of variance;  $p<0.001$ ,  $DF=3$ , Chi-square=24.3. SPL4 vs S1 and SPL3 vs S1:  $p<0.001$ ).

760

761 **Figure 4. Spiking neural network (SNN) design and characterization.** **A**, Schematic of the  
762 procedure used to create a library of SNNs. Starting from the Izhikevich model implemented on the  
763 FPGA and a library of 34 BNNs with a large spectrum of activity, we tuned the mean value of the  
764 synaptic weight distribution to obtain and select from a collection of SNNs (SNN library, comprising  
765 27 different configurations). **B**, Representative 20 s-long raster plots of different BNNs showing  
766 different NB rates. **C**, Left, distribution of excitatory synaptic weights from the 27 SNNs. In red, the  
767 slower SNN of the library (SNN 1). The blue arrow indicates the shift of the mean value (from 0.99 to  
768 1.34) of the normal distribution with standard deviation = 0.3 to obtain increasing NBR values. Right,  
769 distribution of inhibitory synaptic weights from the 27 SNNs. In red, the slower SNN of the library  
770 (SNN 1). The blue arrow indicates the shift of the mean value (from -2.02 to -1.02) of the normal  
771 distribution with standard deviation = 0.3 to obtain increasing NBR values. **D**. Representative 20 s-

772 long raster plots of different SNNs showing different NB rates. **E**, Left, cumulative MFR profile for  
773 the BNN library. Right, cumulative MFR profile for the SNN library. **F1**, Comparison between BNN  
774 and SNN libraries in terms of network MFR (i.e., the mean value of all active electrodes for BNN and  
775 neurons for SNN). **F2**, Comparison between BNN and SNN NBR, showing that the SNN library  
776 covers the BNN variability and contains networks with a higher NBR.

777

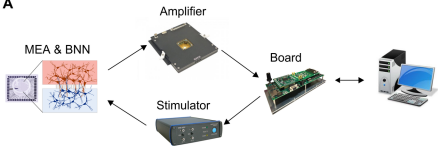
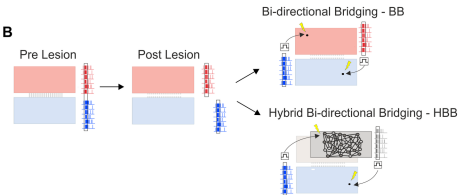
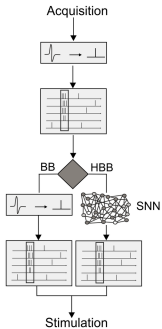
778 **Figure 5. The hybrid bidirectional bridging approach is effective when a neuronal assembly**  
779 **must be replaced.** **A**, Schematic of the experimental protocols. We recorded 20 minutes of  
780 spontaneous activity (S1) followed by laser ablation. Grey-shaded area indicates 20 minutes of no  
781 recording during ablation. Dots represent two hours of no recording after the lesion to obtain stable  
782 activity in both modules and to test different stimulation channels. Then, we recorded 20 minutes of  
783 SPL activity (SPL3) followed by 20 minutes of a hybrid bidirectional bridging (HBB) protocol and  
784 another 20 minutes of spontaneous activity (SPL4). **B1**, Top, 20 s-long raster plot depicting the BNN  
785 bursting activity involving both modules before lesion. Bottom, activity of SNN uncorrelated with the  
786 BNN. The networks are not linked. **B2**, Top, 20 s-long raster plot after lesion showing uncorrelated  
787 bursting activity on BNN modules 1 and 2. Bottom, same as that in B1. **B3**, 20 s-long raster plot  
788 during HBB depicting two hybrid events. The first event on the left was an NB detected on module 1  
789 of the BNN. The detection resulted in a stimulation pulse delivered to 10 excitatory neurons of the  
790 SNN (blue line, bottom). An NB on the SNN was detected 18 ms after the stimulation and triggered  
791 the delivery of a stimulation pulse to module 1 of the BNN (grey line, top). **B4**, 20 s-long raster plot  
792 depicting the uncorrelated activity of BNN modules (top) and SNN network (bottom). **C**, MFR during  
793 the 4 experimental phases was stable (colour code as in panel a: S1: light blue dot; SPL3, SPL4: dark  
794 grey dots; HBB: red dot). No significant difference was found (one-way repeated measures analysis of  
795 variance.  $p < 0.001$ ,  $DF = 3$ ,  $F = 3,16$ ; Bonferroni test: all comparisons with  $p > 0.05$ ). **D1**, CC function  
796 during the 4 experimental phases. Colour code the same as that in panel **A**. Note that during BB, the  
797 cross-correlation function (red) recovers even if not completely with respect to the initial profile (light  
798 blue). **D2**, CC area was highly reduced during the post-lesion phases. The CC area partially recovered  
799 during the BB protocol and collapsed again when stimulation was switched off (one-way repeated

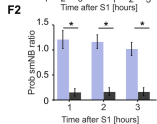
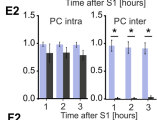
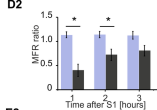
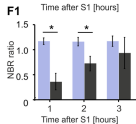
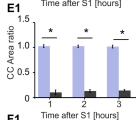
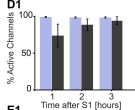
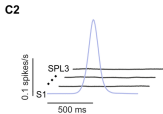
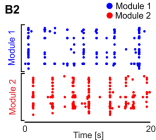
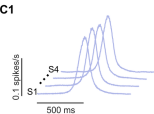
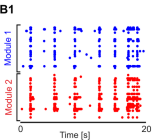
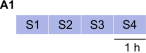
800 measures analysis of variance.  $p < 0.001$ ,  $DF=3$ ;  $F=70,448$ ; S1 vs SPL3:  $p=5.80E-10$ ; S1 vs SPL4  
801  $p=2.72E-09$ ; S1 vs HBB:  $p=9.16E-04$ ; HBB vs SPL3  $p=1.16E-07$ ; HBB vs SPL4  $p=1.02E-06$ ; SPL4  
802 vs SPL3  $p=0.73643$ ). **E1**, NBR did not change during HBB with respect to the S1 phase (one-way  
803 repeated measures analysis of variance.  $p=0.005$ ,  $DF=3$ ;  $F=6,069$ ; S1 vs SPL3  $p=0.02482$ ; S1 vs  
804 SPL4  $p=1$ ; S1 vs HBB  $p=1$ ; HBB vs SPL3  $p=0.01022$ ; HBB vs SPL4  $p=1$ ; SPL4 vs SPL3  
805  $p=0.00674$ ). **E2**, Probability of a single-module NB (Prob smNB) was close to one after the lesion.  
806 During the HBB protocol, the probability was similar to that in the pre-lesion condition (one-way  
807 repeated measures analysis of variance.  $p < 0.001$ ,  $DF=3$ ;  $F=453,439$ ; S1 vs SPL3  $p=4.96E-13$ ; S1 vs  
808 SPL4  $p=1.03E-12$ ; S1 vs HBB  $p=0.22606$ ; HBB vs SPL3  $p=1.94E-12$ ; HBB vs SPL4  $p=4.30E-12$ ;  
809 SPL4 vs SPL3  $p=1$ ).

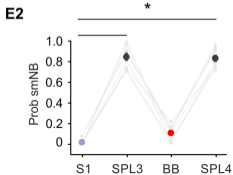
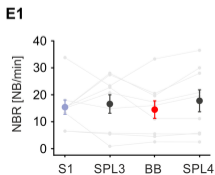
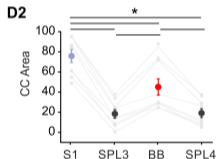
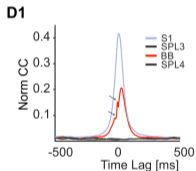
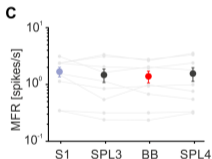
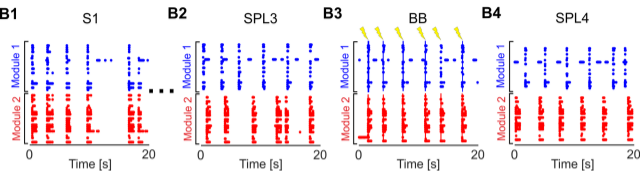
## 810 **Supplemental video**

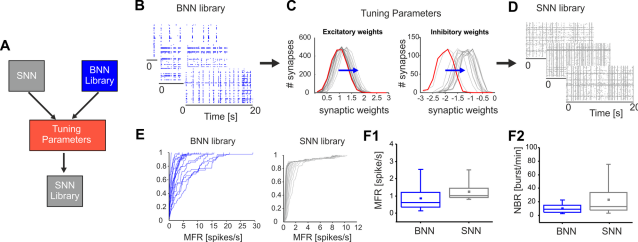
811 **Performing the lesion, related to Figure 2.** Video recorded during a laser ablation of the  
812 connections between two modules (visible on the left and right side) of a bimodular cell  
813 culture. Video recorded on November 16th 2016. Duration 18s. Link:  
814 [https://data.mendeley.com/datasets/bnjckk2kht/draft?a=bb802c21-d45c-4c17-be78-](https://data.mendeley.com/datasets/bnjckk2kht/draft?a=bb802c21-d45c-4c17-be78-34b11d53bed0)  
815 [34b11d53bed0](https://data.mendeley.com/datasets/bnjckk2kht/draft?a=bb802c21-d45c-4c17-be78-34b11d53bed0)  
816

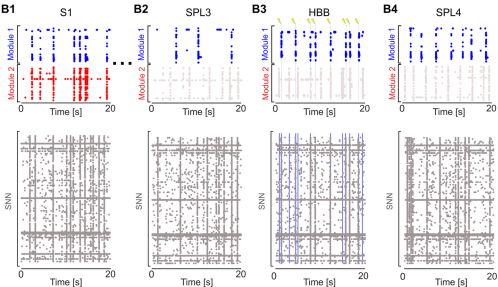
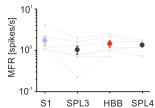
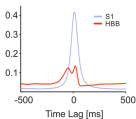
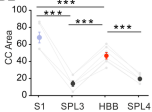
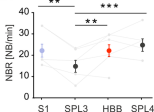
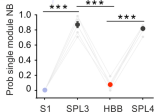


**A****B****C**

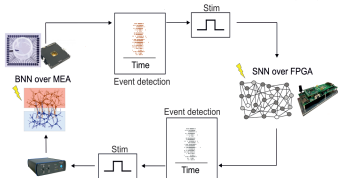






**A****C****D1****D2****E1****E2**

A Biological Neural Network (BNN) dialoguing in closed-loop with a real-time neuromorphic prosthesis based on a Spiking Neural Network (SNN)



In case of a focal lesion in BNN, the neuromorphic prosthesis can implement two reconnection strategies: Bidirectional Bridging (BB) and Hybrid Bidirectional Bridging (HBB)

