



## Chapter 7

# Summary and discussion

### 7.1 Establishment of a neuronal culture on MEAs based model

In chapter 3, in accordance with the first goal of this work, we developed a model of activity in neuronal ensembles based on mouse cortical culture grown on MEAs. Such systems have been widely used since substrate integrated MEAs were first fabricated in the 1980's. However, these past utilizations have been almost exclusively restricted to cultures derived from rat embryos. Consequently, the data provided in this work is interesting with respect to the possibility of using mouse neurons with MEAs which would be desirable on account of the richer molecular biology toolbox available for that model animal. We have shown that mouse cultures indeed develop into an active network and we were able to record both spontaneous and evoked multi channel spiking activity from them. Additionally, the cultures demonstrated several characteristics originally described in the rat systems (reviewed in section 1.2 and also mentioned in section 3.2.1). These include a gradual formation of synaptic connections accompanied by a reorganization of the activity into synchronized network bursts, a clear homeostatic control over firing rates, a modular connectivity organization and a sharpening and narrowing of the bursts over time. This last observation is taken as evidence that the GABAergic system follows a distinct developmental time course compared to its glutamatergic counterpart. Based on these observations we conclude that mouse cultures grown on MEAs are a useful system for studying the dynamics of neuronal ensembles at the network level.

Attempts to induce plasticity in the mouse cultures using a tetanus-based protocol and without addition of any neuromodulators did not produce any significant changes to measures involving either spontaneous or evoked activity. This negative result joins a long list of contradicting reports regarding plasticity in neuronal cultures. Nevertheless, it aligns well with the accepted notion that neuromodulators strongly gate neuronal plasticity (reviewed in section 1.3.1). Thus we regard this negative result as an added motivation to supplement

classical cortical cultures with neuromodulator signalling functional  In this chapter, we also took the extra step of attempting the same plasticity protocol with dopamine present in the media during the tetanus induction epoch and subsequently washed away. The reason behind this particular pattern of application is that dopamine (and neuromodulators in general) is known to have both a direct effect on network dynamics as well as a plasticity effect which persists after the agonist had been removed. Since the experiment was concerned with plasticity, we elected to remove the dopamine prior to the post-induction measurements so that the plasticity effect would not be confused with the direct one. This addition of dopamine represents a novel paradigm that had not been explored in neuronal culture before and interestingly resulted in significant changes to both the evoked and spontaneous activity measures. This result serves as another demonstration that neuromodulators constitute an important part of the system and they are required to observe the full range of forebrain circuit dynamics. Unfortunately, not much may be taken from these results regarding the precise interactions of dopamine with network and synaptic activity because such manual pipetting of the agonist on and off is not conducive to fine tuned associations between these processes. Additionally, this application method does not assure that the dopamine has been completely removed (because enough media from the same culture dish is available for just a single washing step) and might also induce activity effects related to the solution exchange itself. Thus these results highlight the current experimental need for a high precision programmatic solution exchange system operating under continuous flow (to avoid effects associated with the solution exchange itself) and support the endeavour undertaken in this Ph.D work .



As described above, the mouse cultures were shown to develop a characteristic network activity profile and thus to be useful in studying activity dynamics and plasticity. Nevertheless, they seemed to follow a different developmental time course compared to reports from rat cultures. This was mainly to do with a delay in synaptic maturation. In the case of our mouse cultures, the appearance of network activity was clearly characterized by an initial time frame where the MEA channels showed tonic firing with very low levels of correlation and virtually no network bursts. Significant correlations arose only several days later. According to developmental studies of rat cultures the activity in those cases is characterized by significant correlations immediately upon its appearance (see cited references in section 3.2.1). It also seems to be more influenced by synaptic drive because the mean firing rate and the burst rate are correlated and increase until the 4<sup>th</sup> week *in vitro*. In the case of the mouse cultures, even after the appearance of correlations, the activity levels remained constant implying a dominance of intrinsic excitability and homeostasis over synaptic drive. The delayed maturation in mouse cultures is also manifested by the complete lack of superbursts, which were abundant in rat cultures. These high intensity network events are considered to result from a temporary imbalance between excitation and inhibition present in early phases of development in rat, but apparently not mouse, cultures (This argument is expanded in


section 3.2.1). Since the development of neuronal culture is considered to represent, to a certain extent, the cortical development just following birth, above noted differences might reflect a distinction between how the nervous system initially organizes in mouse and in rat. It is surprising that such similar species exhibit such marked differences. Whether these differences serve a functional purpose or merely comprise a genetic jitter which will be overridden by experience dependent organization which occurs later in development remains an open question.

## 7.2 Establishment of a microfluidics based neuronal culture system

In chapter 4, in accordance with the second goal of this work, we were able to establish both long term macro- and micro-cultures in microfluidic devices of the relevant geometries. We found that, in accordance with reports from literature, growing such cultures was challenging as compared to standard ones on open surfaces and required tweaking of the culture, surface and incubation parameters as well as treating of the device materials (PDMS extraction). The micro-cultures were particularly challenging and developed properly only at high densities and when the device design was modified to include support cultures enriching the media with conditioning factors.

The cultures were able to develop inside the microfluidic devices for over 3 weeks when kept in static conditions. However, maintaining their viability under flow proved to be extremely tricky as it caused them to degenerate within several hours. This degeneration occurred over the entire flow range available to our flow system. A quantitative analysis of the degeneration showed that by using conditioned media for the flow, the degeneration was slowed down significantly and that the rate of degeneration was highly correlated with the levels of conditioning. Varying the flow rate across two orders of magnitude did not, however, correlate with the viability in any way. To explain these results we propose that that the deleterious effects of flow are mediated by removal of conditioning factors. based on this premise, conditioned media extends the viability by partially replacing the removed factors. The lack of sensitivity to flow rate is explained by the fact that even minuscule flow is enough to compete with diffusion and remove cell-secreted factors so it is plausible that any level of convection dominant flow would induce the same effect. One way of assessing the dominance of convection is by comparing the average flow velocity to the expected translation of a particle per time unit given a diffusive regime. Thus, for example, a neurotransmitter sized molecule would traverse  $x = 2\sqrt{t \cdot D} = 40\mu m$  in 1 second with  $D = 400 \frac{\mu m^2}{s}$  [149] purely due to diffusion. In this sense, our lowest flow velocity ( $40 \frac{\mu m}{s}$ ) is comparable to diffusion of small neurotransmitter molecules. However, in the literature, the convection-diffusion balance is normally expressed using a different measure, the Peclet number. This measure formally refers to the length of travel down a microfluidic channel

that is required for two laminar streams flowing in it side by side to achieve full mixing via diffusion [174]. The Peclet number provides this length as multiples of channel width (note that it is a dimensionless measure). This number is generally used to quantify the convection-diffusion balance even though it does not have a formal theoretical meaning in some of the contexts, such as the question of removal of secreted factors by convection which is addressed here. The Peclet number for our slowest flow regime is  $P_e = \frac{L \cdot u}{D} = 150$  where  $L = 1500 \mu m$  is the channel width and  $u = 40 \frac{\mu m}{s}$  is the average velocity. This value is still somewhat greater than in previous studies where long term neuronal culturing under flow was achieved (see sections 1.5.2 and 4.1) where the Peclet number was  $P_e < 50$ . This could explain the fact that even in our slowest flow regime, the cultures' degeneration rate was greater than control. Another important detail regarding these previous studies is that the flow in all of them was gravity fed and fully on-chip which means that it was very smooth. It is important to take into account that our flow system may be characterized by a more jerky type of flow because of oscillations originating in the PID control or due to vibrations being transmitted to the cells through the long external tubing. It is plausible that the conditioning processes that are responsible neuronal viability require a fine temporal arrangement of factors, e.g., a stable gradient of growth factors or a precise acing of ECM proteins around an extended neurite. In this case it possible that even a seemingly minor property like flow smoothness could play a big role in the viability. We believe that it would be worthwhile to perform further, more systematic examinations of the effect of different flow regimes on neuronal viability as it will facilitate our understanding of such trophic volume transmission processes which remain poorly understood. These processes would need to be taken into account if long term neuronal survival is to be achieved and they bear relevance to the type of neuroscience experiments that may be performed with the microfluidics approach. 

In this work, considerable focus is directed towards justifying the notion that flow primarily interferes with factor concentrations. This is due to the fact that this narrative contradicts the current perceptions in the microfluidics field which attribute a great degree of shear stress sensitivity to neurons. Morel et. al. specified a need for shear-free agonist delivery but did not check what exactly the impact of shear is [106], Wang et. al. reported a shear dependent retraction of neurites [105] and Liu et. al. reported that a compound isolated from brain tissue confers shear protection to cultured neurons [107]. Although we do not deny the observations made in these studies we claim that their interpretation is somewhat mis ng. Attributing deleterious effects directly to shear implies a physical damage, e.g., tearing holes in the membrane or pulling the cell off the surface. However, as unique as they may be, neurons are still, basically, adherent eukaryotic cells composed of standard universal building blocks (e.g., lipid bi-layer). Thus it is hard to conceive that they would be more prone to physical damage than any other eukaryotic cell. Studies on detachment of adherent eukaryotic cells from the surface due to shear forces report that shear rates 2-3 orders of magnitude greater than the ones typically used in microfluidic neuronal

studies are required to tear the cells off the surface (e.g., [175]). Thus it is very unlikely that physical damage is inflicted to the cells. Nevertheless, there is a viable option is that shear activates stretch receptors which in turn initiate an active cell death process. Indeed such shear sensors have been identified before, but studies involving them usually report a correlation between shear rates and the cellular phenotype [176, 177] which is absent from our results. Overall, untangling pure physical shear effects from those involving localized changes to factor concentrations is very hard to do experimentally. Despite this ambiguity, a major focus is given shear stress as a driver of neuronal death or even of other induced cellular phenotypes (e.g., [176]). This focus probably has a lot to do with the ease at which shear may be defined and measured compared to changes in localized factor concentration. The data provided above serve as a reminder that the latter type of effects are just as or even more likely to play a role.

### 7.3 Activity under flow

In chapter 5, in accordance with the third goal of this work, we developed a protocol whereby the cultures exhibited stable spiking activity under flow. To develop the protocol we monitored the spiking activity of cultures under steady microfluidic flow in devices bonded to commercial MEAs. The final working protocol consisted of a culture, in the 4<sup>th</sup> week *in vitro*, under flow with media from the same culture dish (dubbed ‘self media’). However, the process of developing this protocol we realized that the effects of the flow on the activity seemed to follow exactly the same principles that were uncovered by the viability studies discussed in the previous section, namely, that the flow can cause a disruption to the activity which is completely determined by changes to the chemistry around the cells and not at all by shear stress. The observations which led to this conclusion are as follows: Under fast flow on younger cultures (3<sup>rd</sup> week *in vitro*), the activity suffered a disruption consisting an immediate loss of the stimulation response as well as a sharp de-synchronization of the spontaneous activity which was then gradually silenced altogether. Under very slow flow ( $u = 3 \frac{\mu m}{s}$  and  $P_e = 10$ ) this disruption was completely avoided. Finally, the fast flow experiments were repeated with a semipermeable membrane positioned  $100 \mu m$  above the cells and separating them from the flow. Even though the membrane was shown to only allow negligible shear underneath (much smaller than even in the case of the slow flow above), it did nothing to prevent the activity disruption. By calculating the expected diffusive flux through the membrane assuming that the flow prevents concentrations above it from building up, we were able to show that the above immediate effects on the activity are consistent with the removal of neurotransmitter sized molecules purely by diffusive transport through the membrane (see expanded arguments in section 5.3.2). The final protocol, where the activity was maintained in older cultures, did not require any shear reduction measures and the key was to use self media as described above. Using mismatched media and particularly

media from younger cultures resulted in a disruption very similar to the one reported above for younger cultures.

These results stand shoulder to shoulder with the interpretations of the viability data assessed in the previous section because they both show that the concentrations of conditioning factors are the main consideration to be taken into account when designing such systems. The conditions where activity under flow was maintained were markedly different from the ones that gave the best results in the viability assays. This newer protocol based on the activity data could therefore potentially improve the viability yet further. However, we would like to note that even with most successful protocol where the activity was generally maintained we still observed instability developing over time implying that long term viability was not achieved. An important point that needs to be taken into account is that the extrasynaptic environment is not uniform and static as some idealized caricatures of neural tissue may suggest but rather spatially and temporally complex as it is constructed out of an assembly of ongoing localized secretion events. The calculations in section 5.3.2 showed that fast flow with media whose chemical profile differs substantially from the one present around the cells can act as a strong sink perturbing the concentration around the cells purely due to diffusion. Our results demonstrated that this sink had catastrophic effects even when the flow stream was located  $100\mu m$  away from the cells (i.e., over the membrane). With this in mind, it is very plausible that direct flow, where the stream runs just on top of the culture, flattens the spatial richness of the signalling and imposes a degenerate uniform spatial profile which directly follows the concentrations present in the stream. Thus it would not be surprising if rapid flow, which strips the tissue of its ability to control its own surroundings, will not be conducive to long term viability under any conditioning formulation.

In this context it is worthwhile mentioning that Wagenaar et. al., in their influential study about activity dynamics in neuronal culture [26], mentioned that even small physical perturbations to the culture dish (e.g., moving it across the room) can induce strong activity disturbances which last minutes. The immediate interpretation provided by the authors was that the action of sensitive stretch receptors can generate a lasting modulation of the network dynamics. Our findings show this to not be the case and that, in fact, activity disruption associated with physical perturbations to the culture dish are caused by a temporary mixing of the culture medium which leads to a disruption of factor concentration patterns around the cells.

Despite being pessimistic about the prospect of long term viability under rapid flow, we find that measurement of network activity in these conditions might actually comprise a useful paradigm for studying the importance of extrasynaptic signalling. Even though the richness of volume transmission mechanisms has been receiving increasing attention recently, the fundamental paradigm is still that the functional identity of the network is stored in its synaptic connections and that extrasynaptic processes play only a supporting role. The standard way of performing biological investigations which is highly reductionist

and tends to pay attention to a single bio-molecule at a time, makes it hard to critique the above assertion. However, our system, where the flow essentially abolishes the extrasynaptic signalling, may be used to observe how the involvement of the extrasynaptic compartment is involved in network function as a whole. In section 5.5 we argued, by taking into account the time scales of synaptic processes, that the flow does not interfere with phasic synaptic signalling but rather only disrupts intrinsic volume transmission. This argument further strengthens the notion that rapid microfluidic flow may be used to specifically target the extrasynaptic signalling.

The results provided in this work are already interesting with respect to the link between volume transmission and network function. For example, we find it is surprising that the effects of flow, when the media was not matched, were so profound and seemed to drive the network outside its functional regime (inability to maintain any level of activity and abolishment of the stimulation response). This promotes the view that the fast synaptic currents in fact only modulate the neuronal activity on top of a much stronger signal that is carried by the volume transmission. In that case the functional properties of the circuit were not restored even after a few hours under flow. This demonstrates that the neurons lack other homeostatic mechanisms (e.g., intrinsic or synaptic) that can operate within hours to restore the activity. An accepted paradigm in contemporary neuroscience is that neuronal activity is governed by a balance between excitation and inhibition. These results raise the possibility that this balance is strongly dependent on volume transmission processes. Further information is provided by the experiments with old cultures and self media (i.e., when the media was matched and the activity was maintained). We argued that in this case the flow abolished the active extrasynaptic signalling but kept the concentrations generally in the working range. Under these conditions the fundamental functional identity of the circuit was maintained in the sense that the activity measures that we used as well as the structure of the correlation matrix were roughly maintained (figures 5.8 and 5.7). This supports the original view that intrinsic volume transmission only plays a supporting role with regards to network function. However, these observations were made with only crude measures of functionality and so we propose that, given a more advanced experimental design and analysis, these type of manipulations could provide important insight as to the role of the extrasynaptic environment. In this context it is worth mentioning that the latter experiments (i.e., where the activity was maintained) still exhibited a doubling in the intensity of the network stimulation response and an increase in its length. This could point to an interruption in feedback mechanisms operating through phasic volume transmission to control neuronal excitation. Such mechanisms were observed for GABA [178], adenosine [62] and ATP [63]. This could suggest that indeed some of the network activity stabilization mechanisms which operate via phasic volume transmission have been compromised by the flow.

## 7.4 The phasic neuromodulation model

In chapter 6 we delivered the declared goals of this Ph.D by putting together all the advances made in the preceding chapters and establishing a system for rapid solution exchange to an entire neuronal culture at time scales compatible with phasic neuromodulation. Achieving these time scales required using a microculture because sweeping the interface across a macroculture would have been too slow. However, before performing the work it was unclear whether the microcultures, which comprised just 400-500 cells, would indeed function as the macrocultures did. In this chapter we showed that the microcultures are well confined spatially (i.e., do not extend neurites out of the microwells), that they develop well for over 3 weeks *in vitro*, that they produced spontaneous and evoked spiking activity and that they can be made to maintain stable activity under flow by using the same protocol that was effective for the macrocultures. We further constructed a finite element fluid dynamics model of the agonist pulsing and used it to show that the concentration transient at the bottom of the microwell adheres to the required time scales. We demonstrated how this model may be used to obtain a detailed spatial profile of the concentration transients and to design other pulsing systems that meet bespoke criteria. Beyond the model based validation, we directly demonstrated adherence to the required time scales by pulsing glutamate and monitoring the excitatory response.

The establishment of a functional agonist pulsing system allowed us to design and run an experimental paradigm modeled after the Izhikevic thought experiment (section 1.3.2). The experiment consisted coupling of dopamine pulses to electrical stimulations that induce a reverberatory network response. The stimulation responses were monitored before and after the dopamine coupling epoch to assess whether lasting changes had been induced (i.e., LTP/LTD). Interestingly, the dopamine coupling induced an immediate significant depression in the stimulation responses but this effect disappeared as soon as the coupling was concluded. As described in section 1.3.1 such direct modulation of the network activity by dopamine is a known phenomena. However, it is completely absent from the Izhikevic model which assumes that the effect of dopamine is just to gradually modulate the synaptic strength. We find that this discrepancy is a great demonstration of our system may be used to ground computational theories related to neuromodulation to experimental facts. In virtue of the fine temporal control offered by our pulsing system, the observed direct response could potentially be avoided by increasing the delay between the stimulations and the dopamine reward. This would be compatible with the notion of distal reward (see sections 1.3.2 and 6.5) and could promote a scenario more compatible with the Izhikevic experiment. Nevertheless, it is currently unknown whether the processes of direct modulation and plasticity are indeed independent or if they interact in some way. Our system offers a the means of exploring these questions.

The above-mentioned results of the pulsing study contradict those obtained in the plas-



ticity experiments performed in section 3.4 with bath application of dopamine. Those earlier experiments showed activity modifications which persisted after washing away the agonist. Nevertheless, the later results have been produced using the superior microfluidic based pulsing system where the concentrations levels of the agonist are accurately controlled and effects associated with the solution exchange are avoided through use of continuous flow. We are therefore inclined to believe that the earlier results are an artefact associated with residual dopamine remaining after the washing or with the washing step itself.

#### 7.4.1 Future use of the model and concluding remarks

It is the nature of scientific progress that once you embark on what seems a desolate path, you find that it leads to a rich web of trails traversed by many other explorers. Thus I could not, should not and would not predict all the multitude of ways the system presented here could be applied. From our vantage point, this system was originally conceived with the intention of studying how the timing and concentrations of neuromodulators interact with plasticity in neuronal circuits. However, this path cannot proceed without even observing plasticity in the first place, a way point that we did not reach with the time frame of this work. As reviewed in section 3.4 plasticity in neuronal cultures is a controversial subject and a standard protocol for achieving it has yet to arise. It is commonly argued that since neuronal cultures mature without any form of natural input they degenerate into over-connectedness which makes it harder to induce plasticity. As we mentioned in section 3.3, these cultures exhibit a characteristic network response profile which seems to be fixed regardless of the locus of stimulation. This fact suggests that there is a certain connectivity pattern that is invoked whenever the network is activated. It is our interpretation that by coupling dopamine pulses to stimulation responses we were simply reinforcing an overused circuit where the plastic connections along it had already been saturated. We therefore suggest a next step where we would apply a similar experimental paradigm but reinforce a subset of stimulation responses which contained rare events, e.g., propagation of spiking activity to an electrode which infrequently participates in the response. To achieve this the system would need to be setup to administer pulses in feedback from the activity.

This system was designed to generate rapid agonist pulses across an entire neuronal circuit and, to our knowledge, this is the first demonstration of such a capability with fast kinetic control. In the previous section, we proposed that the system may operate as switch, capable of turning off the intrinsic volume transmission in the tissue. These manipulations are just the tip of the iceberg of what could potentially be achieved with microfluidics technology. One could potentially envisage a design comprising an array of delivery channels where different agonists would be applied in a temporally and spatially controlled manner. Such a system could potentially capture the spatial complexity of localized intrinsic volume transmission processes and provide neuroscience with a valuable tool of investigating information processing in the extrasynaptic compartment, a major part of the system so

far neglected due to lack of experimental accessibility. Exploration of such multi channel, high resolution agonist delivery systems has already begun [179]. We hope that our work will provide valuable information as to how such systems may be combined with neuronal culture. The sky is the limit.

“All that we know about the future is that it is going to be different.” Peter Drucker.

## Appendix A

# Appendix

### A.1 MEA Data sheets

Some text.

### A.2 Parameters of Comsol model

Consider a section of tube of length  $x$  with dimensions as lis

Some more text.

# Bibliography

- [1] S. Hameroff and R. Penrose, “Consciousness in the universe: A review of the orch or theory,” *Physics of life reviews*, vol. 11, no. 1, pp. 39–78, 2014.
- [2] S. Hagan, S. R. Hameroff, and J. A. Tuszyński, “Quantum computation in brain microtubules: Decoherence and biological feasibility,” *Physical Review E*, vol. 65, no. 6, p. 061901, 2002.
- [3] K. E. R., *Principles of neural science*. McGraw-Hill, 2013.
- [4] A. Volterra and J. Meldolesi, “Astrocytes, from brain glue to communication elements: the revolution continues,” *Nature Reviews Neuroscience*, vol. 6, no. 8, pp. 626–640, 2005.
- [5] S. Oláh, M. Füle, G. Komlósi, C. Varga, R. Báldi, P. Barzó, and G. Tamás, “Regulation of cortical microcircuits by unitary gaba-mediated volume transmission,” *Nature*, vol. 461, no. 7268, pp. 1278–1281, 2009.
- [6] A. Berényi, Z. Somogyvári, A. J. Nagy, L. Roux, J. D. Long, S. Fujisawa, E. Stark, A. Leonardo, T. D. Harris, and G. Buzsáki, “Large-scale, high-density (up to 512 channels) recording of local circuits in behaving animals,” *Journal of neurophysiology*, vol. 111, no. 5, pp. 1132–1149, 2014.
- [7] V. Emiliani, A. E. Cohen, K. Deisseroth, and M. Häusser, “All-optical interrogation of neural circuits,” *The Journal of Neuroscience*, vol. 35, no. 41, pp. 13917–13926, 2015.
- [8] L. Lossi, S. Alasia, C. Salio, and A. Merighi, “Cell death and proliferation in acute slices and organotypic cultures of mammalian cns,” *Progress in neurobiology*, vol. 88, no. 4, pp. 221–245, 2009.
- [9] P. G. Nelson, “Nerve and muscle cells in culture,” *Physiological reviews*, vol. 55, no. 1, pp. 1–61, 1975.
- [10] M. A. Dichter, “Rat cortical neurons in cell culture: culture methods, cell morphology, electrophysiology, and synapse formation,” *Brain research*, vol. 149, no. 2, pp. 279–293, 1978.

- [11] D. Mazia, G. Schatten, and W. Sale, “Adhesion of cells to surfaces coated with polylysine. applications to electron microscopy,” *The Journal of cell biology*, vol. 66, no. 1, pp. 198–200, 1975.
- [12] R. W. Wiertz, *Regulation of in vitro cell-cell and cell-substrate adhesion*. PhD thesis, Universiteit Twente, 2010.
- [13] C. R. Easton, K. Weir, A. Scott, S. P. Moen, Z. Barger, A. Folch, R. F. Hevner, and W. J. Moody, “Genetic elimination of gabaergic neurotransmission reveals two distinct pacemakers for spontaneous waves of activity in the developing mouse cortex,” *The Journal of Neuroscience*, vol. 34, no. 11, pp. 3854–3863, 2014.
- [14] T. Morrow, M.-R. Song, and A. Ghosh, “Sequential specification of neurons and glia by developmentally regulated extracellular factors,” *Development*, vol. 128, no. 18, pp. 3585–3594, 2001.
- [15] D. Benson, F. Watkins, O. Steward, and G. Banker, “Characterization of gabaergic neurons in hippocampal cell cultures,” *Journal of neurocytology*, vol. 23, no. 5, pp. 279–295, 1994.
- [16] G. Wallach, J. Lallouette, N. Herzog, M. De Pittà, E. B. Jacob, H. Berry, and Y. Hanein, “Glutamate mediated astrocytic filtering of neuronal activity,” *PLoS Comput Biol*, vol. 10, no. 12, p. e1003964, 2014.
- [17] D. Eytan, A. Minerbi, N. Ziv, and S. Marom, “Dopamine-induced dispersion of correlations between action potentials in networks of cortical neurons,” *Journal of neurophysiology*, vol. 92, no. 3, pp. 1817–1824, 2004.
- [18] M. Kaufman, M. A. Corner, and N. E. Ziv, “Long-term relationships between cholinergic tone, synchronous bursting and synaptic remodeling,” *PLoS One*, vol. 7, no. 7, p. e40980, 2012.
- [19] C. C. Swanwick, N. R. Murthy, Z. Mtchedlishvili, W. Sieghart, and J. Kapur, “Development of  $\gamma$ -aminobutyric acidergic synapses in cultured hippocampal neurons,” *Journal of Comparative Neurology*, vol. 495, no. 5, pp. 497–510, 2006.
- [20] D. H. Hubel, “Tungsten microelectrode for recording from single units,” *Science*, vol. 125, no. 3247, pp. 549–550, 1957.
- [21] N. Herzog, M. Shein-Idelson, and Y. Hanein, “Optical validation of in vitro extracellular neuronal recordings,” *Journal of neural engineering*, vol. 8, no. 5, p. 056008, 2011.
- [22] F. O. Morin, Y. Takamura, and E. Tamiya, “Investigating neuronal activity with planar microelectrode arrays: achievements and new perspectives,” *Journal of bioscience and bioengineering*, vol. 100, pp. 131–43, Aug. 2005.

- [23] G. Buzsáki, “Large-scale recording of neuronal ensembles,” *Nature neuroscience*, vol. 7, pp. 446–51, May 2004.
- [24] S. M. Potter, “Distributed processing in cultured neuronal networks,” *Progress in brain research*, vol. 130, pp. 49–62, 2001.
- [25] S. Marom and G. Shahaf, “Development, learning and memory in large random networks of cortical neurons: lessons beyond anatomy,” *Q Rev Biophys*, vol. 35, no. 1, pp. 63–87, 2002.
- [26] D. A. Wagenaar, J. Pine, and S. M. Potter, “An extremely rich repertoire of bursting patterns during the development of cortical cultures,” *BMC neuroscience*, vol. 7, no. 1, p. 1, 2006.
- [27] H. Kamioka, E. Maeda, Y. Jimbo, H. P. Robinson, and A. Kawana, “Spontaneous periodic synchronized bursting during formation of mature patterns of connections in cortical cultures,” *Neuroscience letters*, vol. 206, no. 2, pp. 109–112, 1996.
- [28] M. Chiappalone, M. Bove, A. Vato, M. Tedesco, and S. Martinoia, “Dissociated cortical networks show spontaneously correlated activity patterns during in vitro development,” *Brain research*, vol. 1093, no. 1, pp. 41–53, 2006.
- [29] A. Bikbaev, R. Frischknecht, and M. Heine, “Brain extracellular matrix retains connectivity in neuronal networks,” *Scientific reports*, vol. 5, 2015.
- [30] Z. Li and M. Sheng, “Some assembly required: the development of neuronal synapses,” *Nature reviews Molecular cell biology*, vol. 4, no. 11, pp. 833–841, 2003.
- [31] G. Ramakers, H. Van Galen, M. Feenstra, M. Corner, and G. Boer, “Activity-dependent plasticity of inhibitory and excitatory amino acid transmitter systems in cultured rat cerebral cortex,” *International Journal of Developmental Neuroscience*, vol. 12, no. 7, pp. 611–621, 1994.
- [32] J. van Pelt, P. S. Wolters, M. A. Corner, W. L. Rutten, and G. J. Ramakers, “Long-term characterization of firing dynamics of spontaneous bursts in cultured neural networks,” *IEEE Transactions on Biomedical Engineering*, vol. 51, no. 11, pp. 2051–2062, 2004.
- [33] T. K. Hensch, “Critical period regulation,” *Annu. Rev. Neurosci.*, vol. 27, pp. 549–579, 2004.
- [34] Z. F. Mainen and T. J. Sejnowski, “Reliability of spike timing in neocortical neurons,” *Science*, vol. 268, no. 5216, p. 1503, 1995.
- [35] A. Gal and S. Marom, “Entrainment of the intrinsic dynamics of single isolated neurons by natural-like input,” *The Journal of neuroscience*, vol. 33, no. 18, pp. 7912–7918, 2013.

- [36] A. Gal, D. Eytan, A. Wallach, M. Sandler, J. Schiller, and S. Marom, “Dynamics of excitability over extended timescales in cultured cortical neurons,” *The Journal of Neuroscience*, vol. 30, no. 48, pp. 16332–16342, 2010.
- [37] S. Reinartz, I. Biro, A. Gal, M. Giugliano, and S. Marom, “Synaptic dynamics contribute to long-term single neuron response fluctuations,” *Frontiers in neural circuits*, vol. 8, p. 71, 2014.
- [38] M.-f. Fong, J. P. Newman, S. M. Potter, and P. Wenner, “Upward synaptic scaling is dependent on neurotransmission rather than spiking,” *Nature communications*, vol. 6, 2015.
- [39] Y. Penn, M. Segal, and E. Moses, “Network synchronization in hippocampal neurons,” *Proceedings of the National Academy of Sciences*, vol. 113, no. 12, pp. 3341–3346, 2016.
- [40] O. Levy, N. E. Ziv, and S. Marom, “Enhancement of neural representation capacity by modular architecture in networks of cortical neurons,” *European Journal of Neuroscience*, vol. 35, no. 11, pp. 1753–1760, 2012.
- [41] M. Shein-Idelson, E. Ben-Jacob, and Y. Hanein, “Engineered neuronal circuits: a new platform for studying the role of modular topology,” *Frontiers in neuroengineering*, vol. 4, p. 10, 2011.
- [42] M. Kwiat, R. Elnathan, A. Pevzner, A. Peretz, B. Barak, H. Peretz, T. Ducobni, D. Stein, L. Mittelman, U. Ashery, *et al.*, “Highly ordered large-scale neuronal networks of individual cells—toward single cell to 3d nanowire intracellular interfaces,” *ACS applied materials & interfaces*, vol. 4, no. 7, pp. 3542–3549, 2012.
- [43] K. Kang, G. Kang, B. S. Lee, I. S. Choi, and Y. Nam, “Generation of patterned neuronal networks on cell-repellant poly (oligo (ethylene glycol) methacrylate) films,” *Chemistry—An Asian Journal*, vol. 5, no. 8, pp. 1804–1809, 2010.
- [44] O. Feinerman, A. Rotem, and E. Moses, “Reliable neuronal logic devices from patterned hippocampal cultures,” *Nature physics*, vol. 4, no. 12, pp. 967–973, 2008.
- [45] G. Robertson, T. J. Bushell, and M. Zagnoni, “Chemically induced synaptic activity between mixed primary hippocampal co-cultures in a microfluidic system,” *Integrative Biology*, vol. 6, no. 6, pp. 636–644, 2014.
- [46] T. Honegger, M. I. Thielen, S. Feizi, N. E. Sanjana, and J. Voldman, “Microfluidic neurite guidance to study structure-function relationships in topologically-complex population-based neural networks,” *Scientific Reports*, vol. 6, 2016.
- [47] J.-M. Peyrin, B. Deleglise, L. Saias, M. Vignes, P. Gougis, S. Magnifico, S. Betuing, M. Pietri, J. Caboche, P. Vanhoutte, *et al.*, “Axon diodes for the reconstruction of

- oriented neuronal networks in microfluidic chambers,” *Lab on a Chip*, vol. 11, no. 21, pp. 3663–3673, 2011.
- [48] S. Dauth, B. M. Maoz, S. P. Sheehy, M. A. Hemphill, T. Murty, M. K. Macedonia, A. M. Greer, B. Budnik, and K. K. Parker, “Neurons derived from different brain regions are inherently different in vitro: A novel multiregional brain-on-a-chip,” *Journal of Neurophysiology*, pp. jn-00575, 2016.
  - [49] L. Agnati, M. Zoli, I. Strömberg, and K. Fuxe, “Intercellular communication in the brain: wiring versus volume transmission,” *Neuroscience*, vol. 69, no. 3, pp. 711–726, 1995.
  - [50] K. Fuxe, A. B. Dahlström, G. Jonsson, D. Marcellino, M. Guescini, M. Dam, P. Manger, and L. Agnati, “The discovery of central monoamine neurons gave volume transmission to the wired brain,” *Progress in neurobiology*, vol. 90, no. 2, pp. 82–100, 2010.
  - [51] L. Agnati, G. Leo, A. Zanardi, S. Genedani, A. Rivera, K. Fuxe, and D. Guidolin, “Volume transmission and wiring transmission from cellular to molecular networks: history and perspectives,” *Acta Physiologica*, vol. 187, no. 1-2, pp. 329–344, 2006.
  - [52] F. Ding, J. ODonnell, Q. Xu, N. Kang, N. Goldman, and M. Nedergaard, “Changes in the composition of brain interstitial ions control the sleep-wake cycle,” *Science*, vol. 352, no. 6285, pp. 550–555, 2016.
  - [53] M. Farrant and Z. Nusser, “Variations on an inhibitory theme: phasic and tonic activation of gabaa receptors,” *Nature Reviews Neuroscience*, vol. 6, no. 3, pp. 215–229, 2005.
  - [54] K. H. Taber and R. A. Hurley, “Volume transmission in the brain: beyond the synapse,” *The Journal of neuropsychiatry and clinical neurosciences*, vol. 26, no. 1, pp. iv–4, 2014.
  - [55] K. Matsui and C. E. Jahr, “Ectopic release of synaptic vesicles,” *Neuron*, vol. 40, no. 6, pp. 1173–1183, 2003.
  - [56] M. Hamann, D. J. Rossi, and D. Attwell, “Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex,” *Neuron*, vol. 33, no. 4, pp. 625–633, 2002.
  - [57] P. Cavelier, M. Hamann, D. Rossi, P. Mobbs, and D. Attwell, “Tonic excitation and inhibition of neurons: ambient transmitter sources and computational consequences,” *Progress in biophysics and molecular biology*, vol. 87, no. 1, pp. 3–16, 2005.



- [58] S. Lee, B.-E. Yoon, K. Berglund, S.-J. Oh, H. Park, H.-S. Shin, G. J. Augustine, and C. J. Lee, "Channel-mediated tonic gaba release from glia," *Science*, vol. 330, no. 6005, pp. 790–796, 2010.
- [59] P. Cavelier and D. Attwell, "Tonic release of glutamate by a dnds-sensitive mechanism in rat hippocampal slices," *The Journal of physiology*, vol. 564, no. 2, pp. 397–410, 2005.
- [60] E. O. Mann and I. Mody, "Control of hippocampal gamma oscillation frequency by tonic inhibition and excitation of interneurons," *Nature neuroscience*, vol. 13, no. 2, pp. 205–212, 2010.
- [61] K. Lenk, E. Räisänen, and J. A. Hyttinen, "Understanding the role of astrocytic gaba in simulated neural networks," in *Engineering in Medicine and Biology Society (EMBC), 2016 IEEE 38th Annual International Conference of the*, pp. 6121–6124, IEEE, 2016.
- [62] M. J. Wall and M. J. Richardson, "Localized adenosine signaling provides fine-tuned negative feedback over a wide dynamic range of neocortical network activities," *Journal of neurophysiology*, vol. 113, no. 3, pp. 871–882, 2015.
- [63] J.-m. Zhang, H.-k. Wang, C.-q. Ye, W. Ge, Y. Chen, Z.-l. Jiang, C.-p. Wu, M.-m. Poo, and S. Duan, "Atp released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression," *Neuron*, vol. 40, no. 5, pp. 971–982, 2003.
- [64] M. E. Rice and S. J. Cragg, "Dopamine spillover after quantal release: rethinking dopamine transmission in the nigrostriatal pathway," *Brain research reviews*, vol. 58, no. 2, pp. 303–313, 2008.
- [65] J. G. Veening, P. O. Gerrits, and H. P. Barendregt, "Volume transmission of beta-endorphin via the cerebrospinal fluid; a review," *Fluids and Barriers of the CNS*, vol. 9, no. 1, p. 16, 2012.
- [66] Q. Gu, "Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity," *Neuroscience*, vol. 111, no. 4, pp. 815–835, 2002.
- [67] C. B. Saper, T. E. Scammell, and J. Lu, "Hypothalamic regulation of sleep and circadian rhythms," *Nature*, vol. 437, no. 7063, pp. 1257–1263, 2005.
- [68] J.-C. Zhang, P.-M. Lau, and G.-Q. Bi, "Gain in sensitivity and loss in temporal contrast of stdp by dopaminergic modulation at hippocampal synapses," *Proceedings of the National Academy of Sciences*, vol. 106, no. 31, pp. 13028–13033, 2009.
- [69] S. Yagishita, A. Hayashi-Takagi, G. C. Ellis-Davies, H. Urakubo, S. Ishii, and H. Kasai, "A critical time window for dopamine actions on the structural plasticity of dendritic spines," *Science*, vol. 345, no. 6204, pp. 1616–1620, 2014.

- [70] S. Huang, M. Treviño, K. He, A. Ardiles, R. de Pasquale, Y. Guo, A. Palacios, R. Huganir, and A. Kirkwood, “Pull-push neuromodulation of ltp and ltd enables bidirectional experience-induced synaptic scaling in visual cortex,” *Neuron*, vol. 73, no. 3, pp. 497–510, 2012.
- [71] S. Otani, J. Bai, and K. Blot, “Dopaminergic modulation of synaptic plasticity in rat prefrontal neurons,” *Neuroscience bulletin*, vol. 31, no. 2, pp. 183–190, 2015.
- [72] J. T. Isaac, K. A. Buchanan, R. U. Muller, and J. R. Mellor, “Hippocampal place cell firing patterns can induce long-term synaptic plasticity in vitro,” *Journal of Neuroscience*, vol. 29, no. 21, pp. 6840–6850, 2009.
- [73] H.-C. Tsai, F. Zhang, A. Adamantidis, G. D. Stuber, A. Bonci, L. De Lecea, and K. Deisseroth, “Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning,” *Science*, vol. 324, no. 5930, pp. 1080–1084, 2009.
- [74] S. Ögren, T. Archer, and S. Ross, “Evidence for a role of the locus coeruleus nora-drenaline system in learning,” *Neuroscience Letters*, vol. 20, no. 3, pp. 351–356, 1980.
- [75] M. E. Hasselmo, “The role of acetylcholine in learning and memory,” *Current opinion in neurobiology*, vol. 16, no. 6, pp. 710–715, 2006.
- [76] S. Otani, O. Blond, J.-M. Desce, and F. Crepel, “Dopamine facilitates long-term depression of glutamatergic transmission in rat prefrontal cortex,” *Neuroscience*, vol. 85, no. 3, pp. 669–676, 1998.
- [77] K. M. Tye, J. J. Mirzabekov, M. R. Warden, E. A. Ferenczi, H.-C. Tsai, J. Finkelstein, S.-Y. Kim, A. Adhikari, K. R. Thompson, A. S. Andalman, *et al.*, “Dopamine neurons modulate neural encoding and expression of depression-related behaviour,” *Nature*, vol. 493, no. 7433, pp. 537–541, 2013.
- [78] M. E. Carter, O. Yizhar, S. Chikahisa, H. Nguyen, A. Adamantidis, S. Nishino, K. Deisseroth, and L. De Lecea, “Tuning arousal with optogenetic modulation of locus coeruleus neurons,” *Nature neuroscience*, vol. 13, no. 12, pp. 1526–1533, 2010.
- [79] K. V. Kuchibhotla, J. V. Gill, G. W. Lindsay, E. S. Papadoyannis, R. E. Field, T. A. H. Sten, K. D. Miller, and R. C. Froemke, “Parallel processing by cortical inhibition enables context-dependent behavior,” *Nature Neuroscience*, vol. 20, no. 1, pp. 62–71, 2017.
- [80] W. Schultz, “Updating dopamine reward signals,” *Current opinion in neurobiology*, vol. 23, no. 2, pp. 229–238, 2013.
- [81] E. S. Bromberg-Martin, M. Matsumoto, and O. Hikosaka, “Dopamine in motivational control: rewarding, aversive, and alerting,” *Neuron*, vol. 68, no. 5, pp. 815–834, 2010.

- [82] L. Pinto, M. J. Goard, D. Estandian, M. Xu, A. C. Kwan, S.-H. Lee, T. C. Harrison, G. Feng, and Y. Dan, “Fast modulation of visual perception by basal forebrain cholinergic neurons,” *Nature neuroscience*, vol. 16, no. 12, pp. 1857–1863, 2013.
- [83] W. Schultz, P. Dayan, and P. R. Montague, “A neural substrate of prediction and reward,” *Science*, vol. 275, no. 5306, pp. 1593–1599, 1997.
- [84] B. J. Venton, H. Zhang, P. A. Garriss, P. E. Phillips, D. Sulzer, and R. M. Wightman, “Real-time decoding of dopamine concentration changes in the caudate–putamen during tonic and phasic firing,” *Journal of neurochemistry*, vol. 87, no. 5, pp. 1284–1295, 2003.
- [85] G. W. Arbuthnott and J. Wickens, “Space, time and dopamine,” *Trends in neurosciences*, vol. 30, no. 2, pp. 62–69, 2007.
- [86] M. Sarter, V. Parikh, and W. M. Howe, “Phasic acetylcholine release and the volume transmission hypothesis: time to move on,” *Nature Reviews Neuroscience*, vol. 10, no. 5, pp. 383–390, 2009.
- [87] C. Dugast, R. Cespuglio, and M. Suaud-Chagny, “In vivo monitoring of evoked noradrenaline release in the rat anteroventral thalamic nucleus by continuous amperometry,” *Journal of neurochemistry*, vol. 82, no. 3, pp. 529–537, 2002.
- [88] D. O. HEBB, *THE ORGANIZATION OF BEHAVIOUR*. 1961.
- [89] T. V. Bliss and T. Lømo, “Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path,” *The Journal of physiology*, vol. 232, no. 2, pp. 331–356, 1973.
- [90] N. Caporale and Y. Dan, “Spike timing-dependent plasticity: a hebbian learning rule,” *Annu. Rev. Neurosci.*, vol. 31, pp. 25–46, 2008.
- [91] G.-q. Bi and M.-m. Poo, “Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type,” *The Journal of neuroscience*, vol. 18, no. 24, pp. 10464–10472, 1998.
- [92] J. Brea and W. Gerstner, “Does computational neuroscience need new synaptic learning paradigms?,” *Current Opinion in Behavioral Sciences*, vol. 11, pp. 61–66, 2016.
- [93] N. Frémaux, H. Sprekeler, and W. Gerstner, “Reinforcement learning using a continuous time actor-critic framework with spiking neurons,” *PLoS Comput Biol*, vol. 9, no. 4, p. e1003024, 2013.
- [94] N. Frémaux and W. Gerstner, “Neuromodulated spike-timing-dependent plasticity, and theory of three-factor learning rules,” *Frontiers in neural circuits*, vol. 9, 2015.

- [95] E. M. Izhikevich, “Solving the distal reward problem through linkage of stdp and dopamine signaling,” *Cerebral cortex*, vol. 17, no. 10, pp. 2443–2452, 2007.
- [96] P. Jonas, G. Major, and B. Sakmann, “Quantal components of unitary epscs at the mossy fibre synapse on ca3 pyramidal cells of rat hippocampus,” *The Journal of Physiology*, vol. 472, no. 1, pp. 615–663, 1993.
- [97] S. Hestrin, “Activation and desensitization of glutamate-activated channels mediating fast excitatory synaptic currents in the visual cortex,” *Neuron*, vol. 9, no. 5, pp. 991–999, 1992.
- [98] W. F. M., *Fluid Mechanics*. McGraw-Hill, 2009.
- [99] S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, and G. M. Whitesides, “Laminar flows: subcellular positioning of small molecules,” *Nature*, vol. 411, no. 6841, p. 1016, 2001.
- [100] A. J. Bae, C. Beta, and E. Bodenschatz, “Rapid switching of chemical signals in microfluidic devices,” *Lab on a chip*, vol. 9, no. 21, pp. 3059–3065, 2009.
- [101] A. M. Taylor, M. Blurton-Jones, S. W. Rhee, D. H. Cribbs, C. W. Cotman, and N. L. Jeon, “A microfluidic culture platform for cns axonal injury, regeneration and transport,” *Nature methods*, vol. 2, no. 8, pp. 599–605, 2005.
- [102] A. M. Taylor, D. C. Dieterich, H. T. Ito, S. A. Kim, and E. M. Schuman, “Microfluidic local perfusion chambers for the visualization and manipulation of synapses,” *Neuron*, vol. 66, no. 1, pp. 57–68, 2010.
- [103] R. Renault, N. Sukenik, S. Descroix, L. Malaquin, J.-L. Viovy, J.-M. Peyrin, S. Bottani, P. Monceau, E. Moses, and M. Vignes, “Combining microfluidics, optogenetics and calcium imaging to study neuronal communication in vitro,” *PLoS One*, vol. 10, no. 4, p. e0120680, 2015.
- [104] L. J. Millet, M. E. Stewart, J. V. Sweedler, R. G. Nuzzo, and M. U. Gillette, “Microfluidic devices for culturing primary mammalian neurons at low densities,” *Lab on a Chip*, vol. 7, no. 8, pp. 987–994, 2007.
- [105] C. J. Wang, X. Li, B. Lin, S. Shim, G.-l. Ming, and A. Levchenko, “A microfluidics-based turning assay reveals complex growth cone responses to integrated gradients of substrate-bound ecm molecules and diffusible guidance cues,” *Lab on a Chip*, vol. 8, no. 2, pp. 227–237, 2008.
- [106] M. Morel, V. Shynkar, J.-C. Galas, I. Dupin, C. Bouzigues, V. Studer, and M. Dahan, “Amplification and temporal filtering during gradient sensing by nerve growth cones probed with a microfluidic assay,” *Biophysical journal*, vol. 103, no. 8, pp. 1648–1656, 2012.

- [107] M. Liu, W. Song, P. Li, Y. Huang, X. Gong, G. Zhou, X. Jia, L. Zheng, and Y. Fan, “Galanin protects against nerve injury after shear stress in primary cultured rat cortical neurons,” *PloS one*, vol. 8, no. 5, p. e63473, 2013.
- [108] Y. J. Choi, S. Chae, J. H. Kim, K. F. Barald, J. Y. Park, and S.-H. Lee, “Neurotoxic amyloid beta oligomeric assemblies recreated in microfluidic platform with interstitial level of slow flow,” *Scientific reports*, vol. 3, 2013.
- [109] J. Kumamoto, H. Kitahata, M. Goto, M. Nagayama, and M. Denda, “Effects of medium flow on axon growth with or without nerve growth factor,” *Biochemical and biophysical research communications*, vol. 465, no. 1, pp. 26–29, 2015.
- [110] I. Breskin, J. Soriano, E. Moses, and T. Tlusty, “Percolation in living neural networks,” *Physical review letters*, vol. 97, no. 18, p. 188102, 2006.
- [111] M. A. Corner, J. van Pelt, P. Wolters, R. Baker, and R. Nuytinck, “Physiological effects of sustained blockade of excitatory synaptic transmission on spontaneously active developing neuronal networks an inquiry into the reciprocal linkage between intrinsic biorhythms and neuroplasticity in early ontogeny,” *Neuroscience & Biobehavioral Reviews*, vol. 26, no. 2, pp. 127–185, 2002.
- [112] M. Chiappalone, A. Novellino, I. Vajda, A. Vato, S. Martinoia, and J. van Pelt, “Burst detection algorithms for the analysis of spatio-temporal patterns in cortical networks of neurons,” *Neurocomputing*, vol. 65, pp. 653–662, 2005.
- [113] J. Van Pelt, M. Corner, P. Wolters, W. Rutten, and G. Ramakers, “Longterm stability and developmental changes in spontaneous network burst firing patterns in dissociated rat cerebral cortex cell cultures on multielectrode arrays,” *Neuroscience letters*, vol. 361, no. 1, pp. 86–89, 2004.
- [114] T. K. Hensch, “Critical period plasticity in local cortical circuits,” *Nature Reviews Neuroscience*, vol. 6, no. 11, pp. 877–888, 2005.
- [115] S. Marom and G. Shahaf, “Development, learning and memory in large random networks of cortical neurons: lessons beyond anatomy,” *Quarterly reviews of biophysics*, vol. 35, no. 01, pp. 63–87, 2002.
- [116] D. A. Wagenaar, J. Pine, and S. M. Potter, “Effective parameters for stimulation of dissociated cultures using multi-electrode arrays,” *Journal of neuroscience methods*, vol. 138, no. 1, pp. 27–37, 2004.
- [117] D. Eytan, N. Brenner, and S. Marom, “Selective adaptation in networks of cortical neurons,” *The Journal of neuroscience*, vol. 23, no. 28, pp. 9349–9356, 2003.

- [118] O. Weihberger, S. Okujeni, J. E. Mikkonen, and U. Egert, “Quantitative examination of stimulus-response relations in cortical networks in vitro,” *Journal of neurophysiology*, vol. 109, no. 7, pp. 1764–1774, 2013.
- [119] P. L. Baljon, M. Chiappalone, and S. Martinoia, “Interaction of electrically evoked responses in networks of dissociated cortical neurons,” *Physical Review E*, vol. 80, no. 3, p. 031906, 2009.
- [120] S. Marom, R. Meir, E. Braun, A. Gal, E. Kermany, and D. Eytan, “On the precarious path of reverse neuro-engineering,” *Frontiers in computational neuroscience*, vol. 3, p. 5, 2009.
- [121] L. Cozzi, P. D’Angelo, and V. Sanguineti, “Encoding of time-varying stimuli in populations of cultured neurons,” *Biological cybernetics*, vol. 94, no. 5, pp. 335–349, 2006.
- [122] D. A. Wagenaar, R. Madhavan, J. Pine, and S. M. Potter, “Controlling bursting in cortical cultures with closed-loop multi-electrode stimulation,” *The Journal of neuroscience*, vol. 25, no. 3, pp. 680–688, 2005.
- [123] D. A. Wagenaar, J. Pine, and S. M. Potter, “Searching for plasticity in dissociated cortical cultures on multi-electrode arrays,” *Journal of negative results in biomedicine*, vol. 5, no. 1, p. 1, 2006.
- [124] E. Kermany, A. Gal, V. Lyakhov, R. Meir, S. Marom, and D. Eytan, “Tradeoffs and constraints on neural representation in networks of cortical neurons,” *The Journal of Neuroscience*, vol. 30, no. 28, pp. 9588–9596, 2010.
- [125] M. Chiappalone, P. Massobrio, and S. Martinoia, “Network plasticity in cortical assemblies,” *European Journal of Neuroscience*, vol. 28, no. 1, pp. 221–237, 2008.
- [126] F. Hamilton, R. Graham, L. Luu, and N. Peixoto, “Time-dependent increase in network response to stimulation,” *PloS one*, vol. 10, no. 11, p. e0142399, 2015.
- [127] M. E. Ruaro, P. Bonifazi, and V. Torre, “Toward the neurocomputer: image processing and pattern recognition with neuronal cultures,” *IEEE Transactions on Biomedical Engineering*, vol. 52, no. 3, pp. 371–383, 2005.
- [128] Y. Jimbo, T. Tateno, and H. Robinson, “Simultaneous induction of pathway-specific potentiation and depression in networks of cortical neurons,” *Biophysical Journal*, vol. 76, no. 2, pp. 670–678, 1999.
- [129] J. le Feber, T. Witteveen, T. M. van Veenendaal, and J. Dijkstra, “Repeated stimulation of cultured networks of rat cortical neurons induces parallel memory traces,” *Learning & memory*, vol. 22, no. 12, pp. 594–603, 2015.

- [130] R. Madhavan, Z. C. Chao, and S. M. Potter, “Plasticity of recurring spatiotemporal activity patterns in cortical networks,” *Physical biology*, vol. 4, no. 3, p. 181, 2007.
- [131] G. G. Turrigiano, “Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same,” *Trends in neurosciences*, vol. 22, no. 5, pp. 221–227, 1999.
- [132] I. Vajda, J. Van Pelt, P. Wolters, M. Chiappalone, S. Martinoia, E. Van Someren, and A. Van Ooyen, “Low-frequency stimulation induces stable transitions in stereotypical activity in cortical networks,” *Biophysical Journal*, vol. 94, no. 12, pp. 5028–5039, 2008.
- [133] D. J. Bakkum, Z. C. Chao, and S. M. Potter, “Spatio-temporal electrical stimuli shape behavior of an embodied cortical network in a goal-directed learning task,” *Journal of neural engineering*, vol. 5, no. 3, p. 310, 2008.
- [134] G. Shahaf and S. Marom, “Learning in networks of cortical neurons,” *The Journal of Neuroscience*, vol. 21, no. 22, pp. 8782–8788, 2001.
- [135] J. le Feber, J. Stegenga, and W. Rutten, “Slow electrical stimuli affect connectivity in cultured neuronal networks,” in *2009 4th International IEEE/EMBS Conference on Neural Engineering*, pp. 546–549, IEEE, 2009.
- [136] C. Gonzalez-Islas and J. J. Hablitz, “Dopamine inhibition of evoked ipscs in rat pre-frontal cortex,” *Journal of Neurophysiology*, vol. 86, no. 6, pp. 2911–2918, 2001.
- [137] E. Biffi, F. Piraino, A. Pedrocchi, G. B. Fiore, G. Ferrigno, A. Redaelli, A. Menegon, and M. Rasponi, “A microfluidic platform for controlled biochemical stimulation of twin neuronal networks,” *Biomicrofluidics*, vol. 6, no. 2, p. 024106, 2012.
- [138] T. M. Pearce, J. A. Wilson, S. G. Oakes, S.-Y. Chiu, and J. C. Williams, “Integrated microelectrode array and microfluidics for temperature clamp of sensory neurons in culture,” *Lab on a Chip*, vol. 5, no. 1, pp. 97–101, 2005.
- [139] J. Park, B. K. Lee, G. S. Jeong, J. K. Hyun, C. J. Lee, and S.-H. Lee, “Three-dimensional brain-on-a-chip with an interstitial level of flow and its application as an in vitro model of alzheimer’s disease,” *Lab on a Chip*, vol. 15, no. 1, pp. 141–150, 2015.
- [140] G. Brewer, J. Torricelli, E. Evege, and P. Price, “Optimized survival of hippocampal neurons in b27-supplemented neurobasal, a new serum-free medium combination,” *Journal of neuroscience research*, vol. 35, no. 5, pp. 567–576, 1993.
- [141] H. Romijn, F. Van Huizen, and P. Wolters, “Towards an improved serum-free, chemically defined medium for long-term culturing of cerebral cortex tissue,” *Neuroscience & Biobehavioral Reviews*, vol. 8, no. 3, pp. 301–334, 1984.

- [142] J. Ray, D. A. Peterson, M. Schinstine, and F. H. Gage, "Proliferation, differentiation, and long-term culture of primary hippocampal neurons.," *Proceedings of the National Academy of Sciences*, vol. 90, no. 8, pp. 3602–3606, 1993.
- [143] J. W. Park, B. Vahidi, A. M. Taylor, S. W. Rhee, and N. L. Jeon, "Microfluidic culture platform for neuroscience research," *Nature protocols*, vol. 1, no. 4, pp. 2128–2136, 2006.
- [144] J. W. Park, H. J. Kim, M. W. Kang, and N. L. Jeon, "Advances in microfluidics-based experimental methods for neuroscience research," *Lab on a Chip*, vol. 13, no. 4, pp. 509–521, 2013.
- [145] P. G. Gross, E. P. Kartalov, A. Scherer, and L. P. Weiner, "Applications of microfluidics for neuronal studies," *Journal of the neurological sciences*, vol. 252, no. 2, pp. 135–143, 2007.
- [146] S. Kaech and G. Banker, "Culturing hippocampal neurons," *Nature protocols*, vol. 1, no. 5, pp. 2406–2415, 2006.
- [147] B. Samel, M. K. Chowdhury, and G. Stemme, "The fabrication of microfluidic structures by means of full-wafer adhesive bonding using a poly (dimethylsiloxane) catalyst," *Journal of Micromechanics and Microengineering*, vol. 17, no. 8, p. 1710, 2007.
- [148] P. Nath, D. Fung, Y. A. Kunde, A. Zeytun, B. Branch, and G. Goddard, "Rapid prototyping of robust and versatile microfluidic components using adhesive transfer tapes," *Lab on a Chip*, vol. 10, no. 17, pp. 2286–2291, 2010.
- [149] A. Johnstone, *Microfluidic systems for neuronal cell culture*. PhD thesis, The University of Nottingham, 2015.
- [150] G. A. Banker, "Trophic interactions between astroglial cells and hippocampal neurons in culture," *Science*, vol. 209, no. 4458, pp. 809–810, 1980.
- [151] M. Morel, J.-C. Galas, M. Dahan, and V. Studer, "Concentration landscape generators for shear free dynamic chemical stimulation," *Lab on a Chip*, vol. 12, no. 7, pp. 1340–1346, 2012.
- [152] G. Brewer, M. Boehler, R. Pearson, A. DeMaris, A. Ide, and B. Wheeler, "Neuron network activity scales exponentially with synapse density," *Journal of neural engineering*, vol. 6, no. 1, p. 014001, 2008.
- [153] A. Grabrucker, B. Vaida, J. Bockmann, and T. M. Boeckers, "Synaptogenesis of hippocampal neurons in primary cell culture," *Cell and tissue research*, vol. 338, no. 3, pp. 333–341, 2009.



- [154] G. Goyal and Y. Nam, “Neuronal micro-culture engineering by microchannel devices of cellular scale dimensions,” *Biomedical Engineering Letters*, vol. 1, no. 2, pp. 89–98, 2011.
- [155] I. Mody and R. A. Pearce, “Diversity of inhibitory neurotransmission through gaba a receptors,” *Trends in neurosciences*, vol. 27, no. 9, pp. 569–575, 2004.
- [156] X. Li, W. Zhou, S. Zeng, M. Liu, and Q. Luo, “Long-term recording on multi-electrode array reveals degraded inhibitory connection in neuronal network development,” *Biosensors and Bioelectronics*, vol. 22, no. 7, pp. 1538–1543, 2007.
- [157] J. Clements, “Transmitter timecourse in the synaptic cleft: its role in central synaptic function,” *Trends in neurosciences*, vol. 19, no. 5, pp. 163–171, 1996.
- [158] P. Sah, S. Hestrin, and R. Nicoll, “Tonic activation of nmda receptors by ambient glutamate enhances excitability of neurons,” *Science*, vol. 246, no. 4931, pp. 815–818, 1989.
- [159] M. J. Wall, “Furosemide reveals heterogeneous gaba a receptor expression at adult rat golgi cell to granule cell synapses,” *Neuropharmacology*, vol. 43, no. 4, pp. 737–749, 2002.
- [160] A. Araque, V. Parpura, R. P. Sanzgiri, and P. G. Haydon, “Tripartite synapses: glia, the unacknowledged partner,” *Trends in neurosciences*, vol. 22, no. 5, pp. 208–215, 1999.
- [161] G. Mittleman, D. Goldowitz, D. H. Heck, and C. D. Blaha, “Cerebellar modulation of frontal cortex dopamine efflux in mice: relevance to autism and schizophrenia,” *Synapse*, vol. 62, no. 7, pp. 544–550, 2008.
- [162] P. E. Phillips, G. D. Stuber, M. L. Heien, R. M. Wightman, and R. M. Carelli, “Subsecond dopamine release promotes cocaine seeking,” *Nature*, vol. 422, no. 6932, pp. 614–618, 2003.
- [163] E. C. Dankoski and R. M. Wightman, “Monitoring serotonin signaling on a subsecond time scale,” *Neurobiological circuit function and computation of the serotonergic and related systems*, 2015.
- [164] L. O. Trussell, S. Zhang, and I. M. Ramant, “Desensitization of ampa receptors upon multiquantal neurotransmitter release,” *Neuron*, vol. 10, no. 6, pp. 1185–1196, 1993.
- [165] M. Mayer and G. Westbrook, “The action of n-methyl-d-aspartic acid on mouse spinal neurones in culture,” *The Journal of Physiology*, vol. 361, p. 65, 1985.
- [166] N. Gorelova, J. K. Seamans, and C. R. Yang, “Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex,” *Journal of neurophysiology*, vol. 88, no. 6, pp. 3150–3166, 2002.

- [167] A. Ferron, A. Thierry, C. Le Douarin, and J. Glowinski, "Inhibitory influence of the mesocortical dopaminergic system on spontaneous activity or excitatory response induced from the thalamic mediodorsal nucleus in the rat medial prefrontal cortex," *Brain research*, vol. 302, no. 2, pp. 257–265, 1984.
- [168] E. W. Rodgers, J. J. Fu, W.-D. C. Krenz, and D. J. Baro, "Tonic nanomolar dopamine enables an activity-dependent phase recovery mechanism that persistently alters the maximal conductance of the hyperpolarization-activated current in a rhythmically active neuron," *The Journal of Neuroscience*, vol. 31, no. 45, pp. 16387–16397, 2011.
- [169] P. Seeman and H. H. Van Tol, "Dopamine receptor pharmacology," *Trends in pharmacological sciences*, vol. 15, no. 7, pp. 264–270, 1994.
- [170] E. Richfield, J. Penney, and A. Young, "Anatomical and affinity state comparisons between dopamine d 1 and d 2 receptors in the rat central nervous system," *Neuroscience*, vol. 30, no. 3, pp. 767–777, 1989.
- [171] P. Seeman, "Brain dopamine receptors.," *Pharmacological Reviews*, vol. 32, no. 3, pp. 229–313, 1980.
- [172] H. D. Brown, J. E. McCutcheon, J. J. Cone, M. E. Ragozzino, and M. F. Roitman, "Primary food reward and reward-predictive stimuli evoke different patterns of phasic dopamine signaling throughout the striatum," *European journal of neuroscience*, vol. 34, no. 12, pp. 1997–2006, 2011.
- [173] K. C. Berridge, "The debate over dopamines role in reward: the case for incentive salience," *Psychopharmacology*, vol. 191, no. 3, pp. 391–431, 2007.
- [174] T. M. Squires and S. R. Quake, "Microfluidics: Fluid physics at the nanoliter scale," *Reviews of modern physics*, vol. 77, no. 3, p. 977, 2005.
- [175] E. Décavé, D. Garrivier, Y. Bréchet, B. Fourcade, and F. Bruckert, "Shear flow-induced detachment kinetics of dictyostelium discoideum cells from solid substrate," *Biophysical journal*, vol. 82, no. 5, pp. 2383–2395, 2002.
- [176] Y.-C. Toh and J. Voldman, "Fluid shear stress primes mouse embryonic stem cells for differentiation in a self-renewing environment via heparan sulfate proteoglycans transduction," *The FASEB Journal*, vol. 25, no. 4, pp. 1208–1217, 2011.
- [177] J. Shemesh, I. Jalilian, A. Shi, G. H. Yeoh, M. L. K. Tate, and M. E. Warkiani, "Flow-induced stress on adherent cells in microfluidic devices," *Lab on a Chip*, vol. 15, no. 21, pp. 4114–4127, 2015.
- [178] G. Tamás, A. Lőrincz, A. Simon, and J. Szabadics, "Identified sources and targets of slow inhibition in the neocortex," *Science*, vol. 299, no. 5614, pp. 1902–1905, 2003.

- [179] A. Scott, K. Weir, C. Easton, W. Huynh, W. J. Moody, and A. Folch, “A microfluidic microelectrode array for simultaneous electrophysiology, chemical stimulation, and imaging of brain slices,” *Lab on a Chip*, vol. 13, no. 4, pp. 527–535, 2013.