Closed-loop Optogenetic Stimulation

Master thesis

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# Introduction

## Benefits of applying closed-loop approach in neuroscience

Feedback systems are pervasive throughout nature, and the brain is no exception. From processes occurring on a molecular level to interactions between different anatomical regions, the endogenous input to these systems depends largely on their previous output (Arsiero, Luscher, & Giugliano, 2007).

Learning, for instance, could not occur without feedback. In fact, the extraordinary adaptive nature of the brain can be attributed to its ability to incorporate feedback into its computations. Yet these closed-loop influences are largely neglected in the majority of experimental designs (Arsiero, Luscher, & Giugliano, 2007). Instead, the neural response is ascribed purely to the applied stimulation, whereas the state of the system prior to stimulation is ignored. The accurate examination of a dynamical system such as the brain on a mechanistic level requires taking into account that dynamical systems, as Ilya Prigogine (1984) stated, “carry their history on their backs” and that this history affects all future states (Buzsáki, 2006). Instead of stimulating ‘blindly’, a closed-loop approach allows one to stimulate the system according to its current state (Arsiero, Luscher, & Giugliano, 2007).

“Ideally we would be able to move toward a circuit-engineering approach, in which devastating symptoms of disease are understood to causally result from specific spatiotemporal patterns of aberrant circuit activity relating to specific neuronal populations” (Diesseroth, 2011). Current technologies have limited our understanding of the dynamics in a cellular circuit due to the high-speed at which this activity evolves, complicating the manual application of accurately timed stimulations The temporally precise stimulation that a closed-loop approach offers provides an invaluable tool for delivering effective perturbations to neural systems (Rolston, Gross, & Potter, 2010).

Achieving a better understanding of neural dynamics is not only important in a research setting, but also necessary to improve the design of brain-machine interfaces. For instance, knowledge of the precise manner in which deep brain stimulation (DBS) affects neural circuits to relieve Parkinson symptoms could help minimize side-effects (Mandat, Hurwitz, & Honey, 2006) and optimize the effects of the stimulation. Closed-loop DBS has so far led to promising results, showing a significant increase in the speed and accuracy of stimulation (Santaniello, Fiengo, Glielmo, & Grill, 2011). The open-loop stimulators that are currently most widely used require an operator to set parameters manually (Afshar, Wei, Lazarewicz, Gupta, Molnar, & Denison, 2011). A closed-loop stimulator could alter parameters dynamically where needed and stimulate when necessary.

## Closed-loop control

A control system consists of two main components that influence each other in such a way as to achieve a ‘desired response’ (Khoo, 2000). One of these components is the process that is being controlled, also referred to as the ‘plant’ or simply the ‘process’ in control engineering terms. The other is the system exercising the control over this process, known as the ‘controller’.

In an open-loop control system this influence is unidirectional. The control action is applied without feedback information from the system it is applied to. The response of the system to this stimulus can then be measured (Bishop & Dorf, 2010). This is currently the most widely used method to perturb neural systems in an experimental setting (Rolston, Gross, & Potter, 2010).

In contrast, in a closed-loop system the control is bidirectional in that the controller receives input from the system it controls (Bishop & Dorf, 2010). The controller operates on basis of a ‘control law’, an algorithm that computes the control action based on the signal it receives from the sensor. (Aström & Murray, 2008). In this manner the characteristics of the stimulation, such the timing or type, depends on the system to be stimulated. For instance, the control stimulus could be a reward given in response to a particular behavior of the animal, or it could be electrical stimulation applied to cells when a certain spatiotemporal firing pattern is detected. The change in the neural activity resulting from the stimulation will again be fed back to the controller and will serve as the basis for the subsequent stimulation (Rolston, Gross, & Potter, 2010).

Figure Difference between closed loop and open loop. (Aström & Murray, 2008)

Closed-loop control has been applied and perfected in control engineering for decades. Neuroscientists can benefit from this extensive knowledge by applying a control framework to their experiments (Afshar, Wei, Lazarewicz, Gupta, Molnar, & Denison, 2011).

## Feedback

The concept of feedback forms the core of a closed-loop control system. A controller operates according to a basic feedback loop consisting of sensing, computation, and actuation (Aström & Murray, 2008). Sensors inform the controller of the current state of the system. Information gathered by the sensors is then used as input to algorithms that compute the necessary control action to achieve the desired effect. Finally this control action is carried out by the actuator. The reaction of the system to this action will, again, be recorded by the sensors, and the loop repeats.

The feedback received from the studied process can be applied in either a negative or a positive manner.

Negative feedback is the most commonly applied type of feedback in control systems engineering due to its ability to stabilize systems using corrective actions based on the measured difference between the desired output and the actual output (Bishop & Dorf, 2010). Stimulation can be applied to maintain a certain desired state, for instance to achieve homeostasis, altering the dynamics of a system in such a way to make it suitable for a particular application. In electronics negative feedback is used to induce linear behavior from nonlinear systems (Aström & Murray, 2008). This approach also benefits brain machine interface design where a linear control system can be designed by selectively drawing the required information from the nonlinear components of the brain.

Positive feedback, on the other hand, is rarely utilized in control engineering. Instead of taking the difference between a desired output and an actual output, the output of the system is amplified. This amplified signal is then fed back to the controller, where it is amplified it again. This rapid growth, and the destabilizing effect it has on the system, is the reason positive feedback is often undesired in many engineering applications. Take for instance the case where positive feedback creates excessive amplification on a microphone, resulting in an increasingly higher pitched noise. Interestingly, biological systems make frequent use of positive feedback. In nature this escalating effect tends to either saturate after a certain amount of time, perhaps due to lack of resources necessary for further growth, or it is kept in check by its surroundings, which will dampen it when it reaches a certain threshold, preventing runaway growth (Bishop & Dorf, 2010). The latter case could be described as a positive feedback system coupled to negative feedback systems (Aström & Murray, 2008). This serves as a reminder that naturally biological feedback systems do not exist in a vacuum but are instead inherently embedded in a complex context, coupled to many other feedback systems and dependent on their environment.

A common function of positive feedback in biological systems is the facilitation of switch-like behavior as is seen in the autoregulation of genes (Aström & Murray, 2008). It can also be used to generate rhythms such as oscillations, central pattern generators, or circadian rhythms. Switching behavior can be created through positive feedback by inducing a different state only when a certain threshold is crossed. The existence of a well-defined threshold is made possible by a dynamical process called hysteresis. Hysteresis carries the system from one state to another state far removed from the first, effectively preventing it from jumping back and forth too easily between the states due to noise (Bishop & Dorf, 2010). This kind of system with two separate, clearly defined states is referred to as ‘bistable’ (Berndt, Yizhar, Gunaydin, Hegemann, & Deisseroth, 2009).

A closed-loop control framework for in vivo experiments

Setting up a closed-loop experiment requires a clear formulation of the research question. This will determine not only the neural system to be interacted with in a closed-loop fashion, but also the specific components of the system to be targeted. For instance, you may be interested in the role of inhibitory neurons in a particular process.

Figure : closed-loop system. Green: neural system, Purple: controller.

First, a sensor is needed to collect the output from the studied system (Aström & Murray, 2008). For instance, recording electrodes can observe the activity of the interneurons, both in their natural state, as well as their response to the applied stimulation.

The acquired signal will consist of more than the neural activity of interest. Preprocessing usually consists of filtering and amplifying the incoming signal. The goal of a filter is to ensure that only those parts of the signal that resemble realistic neural activity will be included, removing noise, artifacts and other activity in the signal that does not appear to originate from a biological source. Moreover, the signal has to travel along wires to reach the recording system where it will be processed further. To prevent loss of information along the way the signals can be enhanced using amplification.

Automatic detection techniques can be utilized to extract spikes which can then be clustered in groups according to their most probably origin. Perhaps the spatiotemporal pattern of the activity of theses cells during a particular behavioral event is of interest to the research question. In this case a further selection can be made where only cells consistently active during this event over several trials could be taken into consideration. This significantly reduces the features that will ultimately need to be detected online, making detection more efficient.

 A mathematical model can be used to distinguish the spatiotemporal pattern among these cells. Once this pattern is detected it can be acted on in various ways. Ideally, a certain cell type could be excited or inhibited upon detection of the pattern and the behavioral output as well as the change in neural dynamics picked up by the recording set up can be studied to provide insight into the specific role this type of neuron plays in the neural dynamics underlying a particular event.

## Online stimulation of closed-loop systems

Electrical stimulation is currently the most widely used form of stimulation in closed-loop experiments. However this technique has many limitations (Stanslaski, et al., 2012). The high level of stimulation needed to activate cells far exceeds the amplitude of action potentials generated by neurons, thereby obscuring this natural activity with artifacts from the stimulation. Closed-loop systems experience a related drawback when electrical stimulation is used, namely feedforward interference. This refers to an effect where the electrical activity generated by the stimulation is picked up by the sensors, mistaken for output of the neural system, and subsequently acted upon by the actuator. (Stanslaski, et al., 2012)

Figure : Feedforward interference in a closed-loop control system

Moreover the lack of precision associated with electrical stimulation is also a limiting factor. For instance, all neurons in the target area are activated simultaneously with electrical stimulation, which is problematic for the accurate clustering of spikes. The resulting synchronous discharge will likely result in the activation of cells in surrounding areas as well, making it difficult to separate if the observed effect is due to the activity of the neurons in the targeted area or connected areas (Fujisawa, Amarasingham, Harrison, & Buzsaki, 2008)**.** A similar loss of spatial precision can occur when, due to the lack of control over the stimulation target, axons are stimulated before the soma, possibly leading to simultaneous activation of cells in other areas as well, despite the locally applied stimulation (Histed, Bonin, & Reid, 2009). This poses a problem not just to our understanding of the neural dynamics, but also in a clinical setting, where debilitating side-effects can occur when electrical stimulation spreads beyond the target area (Mandat, Hurwitz, & Honey, 2006). The collective stimulation of non-therapeutic cell-types, even within the target area, can also result in undesirable effects (Aravanis, et al., 2007). Furthermore, in order to improve deep brain stimulation (DBS) therapy we first need to understand the effect that this stimulation has on different cell types, and role that these cell types play in the circuit dynamics (Gradinaru, Mogri, Thompson, Henderson, & Diesseroth, 2009). Due to the lack of cell-specificity electrical stimulation does not lend itself well to such questions.

Although electrical stimulation led to many new insights the need for a more precise form of stimulation was apparent. Francis Crick expressed concern with the black box manner in which neuroscientists interacted with the brain. He argued that, instead of blindly stimulating all cells in a certain region, much more informative answers could be acquired through awareness of the system’s individual components, and interacting with these in a highly specific manner to unveil their role in the observed behavior. “To understand a complex biological system one must be able to interfere with it both precisely and delicately” (Crick, 1999). Crick suggested the use of light to achieve this selective stimulation. At the time this idea seemed far-fetched. Although light sensitive cells were known to exist in other species, it was not deemed possible to export these light sensitive qualities to mammalian neurons. In 2005, however, the Diesseroth lab in Stanford published a paper revealing a successful attempt at doing exactly this (Boyden, Zhang, Bamberg, Nagel, & Diesseroth, 2005). Neurons could now be genetically altered to contain microbial opsin genes coding for ion channels or pumps that responded to light. This technique, referred to as ‘optogenetics’, elevated the study of circuit dynamics to a new level by facilitating cell-type specific stimulation (Miesenbock & Kevrekidis, 2005). Stimulating the brain with light results in a much higher signal-to-noise ratio (Gradinaru, Mogri, Thompson, Henderson, & Diesseroth, 2009), since this novel technique circumvents many of the artifacts associated with electrical stimulation.

Selective activation and inhibition of a specific cell type in vivo during a behavior is a powerful tool to investigate the role of that type of cell in the network coding for that behavior. If the inhibition of these cells leads to a disturbance in the behavior this cell type can be said to be necessary for this behavior. However, the activity of other cell types may be also be required to generate the observed behavior. When the behavior occurs purely from the stimulation of that specific cell type then those cells are said to be sufficient for the behavior (Bernstein & Boyden, 2011). The cell-specificity that optogenetic stimulation provides facilitates these investigations into the role that different cell types play in network dynamics.

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# Signal Acquisition: Recording

Various methods are available to read out optically stimulated activity, these include spatially oriented methods such as fMRI and optical readouts. Fluorescent molecules are used in optical imaging techniques to reveal the structure and function of neural tissue. Fast optical imaging has achieved relatively high temporal precision through high scan rates (Saggau, 2006). Although methods such as optical microscopy are progressing rapidly they are still limited in their application to free moving animals as well as in their access to deep structures (Dugué, Akemann, & Knöpfel, 2012). For the purpose of this review we will therefore focus on electrical recording, which has been the most commonly employed method to read signals from real-time, closed-loop stimulation in behaving animals.

Recording electrodes can pick up action potentials generated by neurons when they are close enough to the active cell to detect the change in voltage. Neural processes often involve thousands of cells, but to record from this many cells would be unattainable with current technology. However recording from a few hundred cells simultaneously with strategically placed electrodes is realistic and can be very informative when studying microcircuits (Buzsáki, 2004).

Single electrodes can be used to measure neural activity, however this limits the precision with which spikes can be identified as belonging to a particular neuron. Multisite recordings offer the advantage of several different ‘perspectives’. For instance, a spike will usually register on several electrodes, allowing better localization of the source (Buzsáki, 2004). Measuring from multiple electrodes allows the simultaneous recording of many neurons, facilitating the study the interaction between cells and the spatiotemporal dynamics of cell assemblies (Harris, Henze, Csicsvari, Hirase, & Buzsáki, 2000)

Inserting electrodes into the brain results in tissue damage. To keep the extent of this damage to a minimum, the diameter of the electrodes must be as small as possible (Buzsáki, 2004). Small electrode size also facilitates more recording sites in small brains, such as those of rodents. A group of four thin wires is called a tetrode. This electrode configuration is advantageous for spike sorting due to its 3D layout. Furthermore, tetrodes are inexpensive to make. Even smaller in size, but significantly more expensive are the commercially available silicon probes. These high-density probes can record from over a hundred channels simultaneously. Additionally, their small size means multiple probes can be inserted in different areas of the brain, allowing cells from various regions to be recorded simultaneously (Royer, Zemelman, Barbic, Losonczy, Buzsáki, & Magee, 2010)

# Preprocessing & signal enhancement

Raw data often contains a considerable amount of noise. Preprocessing techniques serve to increase the signal to noise ratio. A large fraction of this noise can be attributed to artifacts, such as electrical artifacts associated with the recording and stimulation technology (Rolston, Gross, & Potter, 2010). These artifacts generally have a much higher frequency than the neural signal. With a low-pass filter these unnatural frequencies can be removed from the signal. This step is usually performed by an analog circuit on the headstage of the animal in the case of an in vivo recording (Stark, Koos, & Buzsáki, 2012). The data is then directed from the headstage to a hardware system that will digitize the analog signal. Before this occurs however the signal is amplified, preventing a loss of information along the way. At this point the previously filtered wideband signal will often be filtered again. The manner in which this filtering is performed depends largely on the research question. For instance, spikes can be optimally detected in a high frequency signal, therefore when spiking activity is of interest a high-pass filter would be suitable.

Local field potentials (LFPs), on the other hand, are best viewed at a low frequency sampling rate from 1-500Hz (Rampp & Stefan, 2006). The choice of filter depends on the research question at hand. The signal can also be split into two separate streams, one containing high frequency data for spike detection and the other consisting of low frequency activity to monitor local field potentials (Rolston, Gross, & Potter, 2009). Specialized hardware can then be employed for online spike detection. Such hardware, however, is generally priced anywhere from $10,000 – 40,000 (Rolston, Gross, & Potter, 2009). This large investment was previously unavoidable when implementing a closed-loop system online considering the high processor demands of real-time computations.

However, high-end personal computers on the market today are powerful enough to perform real-time filtering and spike detection. Moreover, software offers the advantage of flexibility. For instance, programs can easily be altered when demands change for different tasks (Rolston, Gross, & Potter, 2010). An example of software used for real-time processing of neural data is presented by Zrenner and colleagues, who implemented a signal-processing model in Simulink (Matlab, Mathworks) operating on a computer running a dedicated real-time operating kernel (Mathworks, xPC target) (Zrenner, Eytan, Wallach, Thier, & Marom, 2010).

# Pattern identification & detection

## Feature extraction & selection

Not all parts of the preprocessed signal will be informative in terms of the research question. Feature extraction and selection further prune the signal by detecting the meaningful features and passing only relevant features onto the higher-level detection algorithms.

Feature extraction involves characterizing the preprocessed signals in a manner that is most relevant to your research question. Say you are interested in the occurrence of a certain oscillation during a particular behavioral task. In this case you would want to extract spectral features from the data and focus your analysis on these. If, on the other hand, you seek to find a firing pattern involved in coding for this task it would be useful to examine the change in the spatial pattern of spikes over the time course of the task. Of course you may be interested in the both the temporal and spatial aspects of the data, for instance to reveal the timing with which a certain oscillation affects the activity of a cell assembly, or how the frequency of neural activity changes with task demands. In this case a time-frequency representation of the data would provide the most insight.

Spectral features are popular for online detection due to the computational efficiency with which they can be detected (Bashashati, Fatourechi, Ward, & Birch, 2007). They do not require a computationally heavy model to dynamically extract features and detect a relevant pattern. Instead, the incoming data is compared to a predefined template of the desired spectral phenomenon. When the data matches the template it requires no further interpretation and can therefore be acted upon immediately by a control mechanism, such as optogenetic stimulation.

When the activity to be detected is more complex, such as a spatiotemporal firing pattern that takes place during a certain event, a mathematical model may be necessary to extract the meaningful patterns as they occur (Andersen, Hwang, & Mulliken, 2010). A neural encoding pattern for a certain behavior not only involves a particular static spatial pattern of firing, but the manner in which this pattern evolves over time may in fact be the most informative.

### Spike detection

The detection of spikes is most commonly performed using a simple threshold. The parts of the signal where the amplitude crosses the threshold are identified as spike times (Franke, Natora, Boucsein, & Munk, 2010). A simple feedback loop might only need to detect spikes on one channel, and react accordingly when it detects a spike on this channel. A more selective algorithm could react only to spikes from a certain neuron. Spikes from the same cell usually share common identifying characteristic waveforms and tend to have very similar amplitudes (Franke, Natora, Boucsein, & Munk, 2010). In this case it is possible to define a template beforehand by defining the unique spike waveform generated by the neuron of interest. Stimulation is applied when the waveform of the detected spike matches the template (Stark, Koos, & Buzsáki, 2012).

### Spike sorting

A single action-potential is often picked up by several electrodes, a phenomenon known as the ‘stereo-effect’ (Gray, Wilson, & McNaughton, 2000). Consequently, when measuring from multiple channels simultaneously the detected action potentials will need to be organized according to their source. When electrodes are spaced close together this becomes especially relevant. As mentioned above, the source of the action potential could be identified by waveform and amplitude. However identifying cells purely based on these characteristics unjustly assumes that output from these cells remains identical over time (Buzsáki, 2004). Another method of spike sorting is triangulation, where cells are identified according to their spatial location. This location is deduced from the timing of a given spike as it arrives at different electrodes. That is, if multiple electrodes in close vicinity detect a spike around the same time it could be a single spike originating from one neuron. The electrode that detected the spike first is most likely the one it is closest to, concurrently this will be the electrode that registers the highest amplitude for this spike. The delay and amplitude with which a spike arrives at each of the other electrodes can reveal the distance between each electrode and the active neuron (Franke, Natora, Boucsein, & Munk, 2010). Thus, the comparison of signals from different electrodes can provide spatial information revealing the origin of a detected action potential.

### Dimensionality reduction for feature selection

With each processing step the reconstructed signal gains increasingly more meaning, and becomes easier to interpret. Further selections of relevant activity will improve the performance of the encoding model that is implemented to extract meaningful spatiotemporal patterns out of this data. Only the most informative features should be used as input to the model. Not all cells measured will necessarily be involved in the activity of interest. This selection of the most informative subsets of the data is referred to as reducing the dimensionality of the feature space, or simply: dimensionality-reduction (Bashashati, Fatourechi, Ward, & Birch, 2007). Principle component analysis (PCA) is a popular dimensionality reduction method. PCA can be applied to high dimensional data sets, that, for instance consist of recordings from many channels, or activity from many different neurons and will select only those who’s activity contributes most to the covariance of the group. Neural activity is by no means stationary as signals vary over time. If the activity of multiple neurons fluctuates in a similar manner during the time course of a certain behavioral event, for instance, their fluctuations in activity may be related to this event. Studying the activity of these neurons would then be more beneficial to understanding the underlying mechanisms of the event in question than incorporating the activity of a neuron who’s fluctuations do not seem to be related to the event or to the fluctuations of other neurons. PCA orders the data in this manner, from most important , or covariant, components to the least important, allowing the selection of only those aspects of the data deemed relevant and meaningful for model input (Bashashati, Fatourechi, Ward, & Birch, 2007).

Hampson and colleagues (2012) apply many of the techniques described above in their design of a memory prosthesis. They approach this task by recording from the hippocampus using electrode arrays containing 16 electrodes in total. The signal was amplified using a low-noise amplifier and converted from analog to digital. Spikes were detected using time-amplitude window discrimination, and sorted by waveform characteristics with dedicated hardware. Those cells with waveforms and firing rates resembling the action potential characteristics of CA1 or CA3 pyramidal cells were selected. Of these cells only those that appeared to consistently respond to a specific combination of events within the trial, referred to by Hampson as ‘functional cell types’, were chosen. In this manner the signal was prepared to serve as input for the multiple-input, multiple-output (MIMO) model, which is able to identify and detect patterns in this data.



Figure : Signal processing of input signals to serve as input to the MIMO model (Berger, et al., 2012)

## Pattern identification & detection

We can now turn to finding patterns within this seemingly relevant data. Pattern detection is considered most successful when the patterns of activity found are uniquely involved in the process of interest (van Greven, et al., 2009)

The purpose of employing a closed-loop approach in neuroscience is often either to understand the underlying neural mechanisms of a certain process or to detect the neural activity coding for the process in question and act on it for practical purposes, to control a brain-machine interface, for instance. Modeling can be used to achieve both of these purposes. The former approach requires a parametric model. This type of model seeks to describe the dynamics between the measured input and the output of the system in a bottom-up fashion, basing the internal structure of the model on prior knowledge and known biophysical laws (Khoo, 2000). The Hodgkin & Huxley model takes this approach by describing the dynamics of the flow of ions across the cell membrane taking into account the known biophysical properties cells and ions. The biological interpretability of a parametric model has proven to lend itself very well to furthering our understanding of neural processes through the comparison of model results with experimental results. A drawback of this approach, however, is that the hypothesis we define in the form of a model is limited to our own imagination, leaving little room to learn from the observation of the system. Given the complex and often counterintuitive workings of nonlinear systems we run the risk of overlooking vital aspects of the process inherent in the data and perceiving only what we expect to perceive, biased by the rigid, predefined structure of the model.

Therefore, instead of formulating a hypothesis regarding the causal definition transforming input to output, a nonparametric model does not make assumptions but empirically observes the relationship between the input and output of the system, treating the process in between as blackbox (Khoo, 2000). This type of model takes a top-down approach by only noting the correlations between inputs and outputs from the data, instead of attempting to define the causality between the two. It uses the found correlations to predict the output from a particular input. In contrast to parametric models, non-parametric models are descriptive instead of prescriptive. Non-parametric models do not define a specific mechanism underlying the transformation of an input to an output but provide a generalized mapping of inputs to outputs instead. Although the abstract nature of this approach makes nonparametric models straightforward, widely applicable and scalable, it does complicate the interpretation of the model in terms of biophysically realistic mechanisms (Song & Berger, 2010).

Parametric and non-parametric approaches are not necessarily mutually exclusive. They can also be combined in the same model. This is the approach taken by Hampson et al (2012) who not only strives to gain a better understanding of dynamics between hippocampal regions but also to build a hippocampal prosthesis. To achieve both of these goals Hampson uses variables that are biophysically interpretable within a nonparametric framework that models the larger-scale, nonlinear transitions such as how input spikes from one region result in postsynaptic potentials in another region. The multiple-input, multiple-output model (MIMO) used by Hampson consists of several multiple-input, single-output (MISO) models. The internal structure of the MISO model is based on electrophysiological properties of a single neuron, taking into the consideration the transformation from an input spike train to a synaptic potential, preceding output spikes to after-potentials, intrinsic noise, pre-threshold potential, and a threshold function. However the exact dynamics of each of these components are modeled non-parametrically. A maximum likelihood method is employed to estimate the parameters in this model from recorded data (Song & Berger, 2010).

Next, the models are generated based on the recorded data. Using every the estimated parameter found by the maximum likelihood method would result in a computationally inefficient, overly complex model. Furthermore, not all parameters found in the data will belong to the signal of interest. In fact, a large subset may consist of noise. This can result in over fitting, as the model would primarily be fitting to noise. The process of selecting an optimal set of parameters is referred to as ‘model selection’. Model selection involves several steps. First of all the recorded data is divided into a training set, used for model estimation, and a test set, used to validate the result from the training set. The model is essentially build from the ground up, starting with a model of the Gaussian noise, followed by the addition of feedback terms, which prediction the output spike train based only the previous spike trains. Next inputs are introduced in a sequential manner. Adding too many inputs in this training phase will lead to over fitting on the training data, resulting in a poor fit on the test data. For every input added, a check for over fitting, referred to as cross-validation, is performed. New inputs are added as long as no evidence of over fitting is present. Lastly the cross-kernels are selected. Cross terms are generated for every unique pair of previously selected inputs and selected using the above described sequential, cross-validation approach. A normalized reconstruction of the kernels is then computed to describe the non-linear input-output dynamics of the model in a more intuitive manner (Song & Berger, 2010).

Figure : schematic diagrams of spike train propagation from CA3 to CA1 (A), the MIMO model (B) and the structure of its MISO model components (C). (Berger, et al., 2012)

Finally, assessing goodness-of-fit through the comparison of predicted output spike trains and actual output spike trains validates the chosen MISO models. The inclusion of a noise term results in stochastic outputs from the kernel models. This complicates the comparison of the predicted and recorded outputs to assess goodness of fit, considering outputs are not a linear result of inputs. That is, even identical inputs will result in different outputs every time. Direct comparison methods as mean-square error, are therefore not well suited for this task. Two indirect comparison methods were used instead, the first of which examined the continuous firing probability intensity. The recorded spike train was rescaled by the firing intensity function generated by the model into a stochastic process that summarized the occurred events, their timing, and the intervals between the events during a certain time period, also referred to as Poisson process. This allowed inter-spike intervals to be normalized as independent uniform random variables between 0 and 1. The goodness-of-fit of the model was then evaluated by ordering the intervals between the rescaled spikes from smallest to largest, and plotted against the cumulative distribution function of the uniform density. If all points are located on a 45 degree line within the confidence bounds the model is considered to have a good fit to the data. This method is referred to as the Kolmogorov-Smirnov (KS) test. The second method employs a Gaussian smoothing process to compare the actual and predicted spike trains. The amount of smoothing needed, measured by the smoothing parameter, determines how well the model fits the data. If relatively little smoothing is required to achieve a high similarity between the two spike trains then the fit of the model is evaluated as accurate (Hampson, et al., 2012; Song & Berger, 2010).

This model was used by Hampson to predict CA1 output from CA3 input based on patterns detected in the subset of the data used for training. Rats were trained in a Delayed-Non-match-to-Sample (DNMS) memory task. At the start of a trial one of two levers is extended. The pressing of this lever by the rat is referred to as the sample response (SR). This response is then followed by a delay period. Following the delay the rat is required to push this same lever. This response, also referred to as the non-match response (NR), requires the rat to hold the information regarding the previously pressed lever in working memory over the course of the delay period. The length of the delay period was found to be inversely related to the performance. Short delay periods would result in a significantly better result than their longer counterparts. Data recorded from several sessions was used for the construction of the MIMO model, as described above. The model learns several patterns of ensemble activity, representing various outcomes of the trial. Trials in which the correct lever was still recalled following a long delay period were labeled as represented by ‘strong SR codes’, while trials with a short delay period yet poor performance were identified as having ‘weak SR codes’. The model was then implemented online, in a closed-loop fashion. The length of the trial depended on the type of activity pattern found. Upon detection of a ‘strong code’ during the SR the delay period was lengthened. The detection of a weak code resulted in a shortened delay period. As expected, performance declined when delays were lengthened. However, significantly less so than when this same increased delay was applied in normal trials, where no strong SR code was detected. Similarly, shortening delays for trials with weak SR codes did result in a better performance, however shortening normal trials in the same manner led to significantly better results. The closed loop approach employed here confirmed the functional significance of predicted patterns from the MIMO model.

Hampson took these results a step farther to reveal the necessity of these codes for performance by stimulating in the same temporal pattern as the strong SR codes. When stimulated with a strong code for the incorrect lever performance levels dropped significantly, suggesting that the patterns identified by the model are at least temporally accurate (Hampson, et al., 2012).

Optogenetic stimulation in place of electrical stimulation could considerably improve the spatial accuracy of the stimulated patterns in the experiments described above. Moreover, the patterns can be further refined through the identification of the various roles played by different cell types in the pattern.

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# Stimulation: The optogenetic toolkit

Although optogenetics is a relatively new technique, many control tools have been developed in the form of different opsin expressions. Some will excite the cell upon illumination, and others will inhibit it. Several ‘fast’ opsins with quick recovery times have been engineered for purposes such as high frequency stimulation (Gunaydin, Yizhar, Berndt, Sohal, Diesseroth, & Hegemann, 2010), while others will excite the cell in slow steps, merely bringing it closer to its threshold (Berndt, Yizhar, Gunaydin, Hegemann, & Deisseroth, 2009). This allows a selection of cells from a chosen cell type to have an elevated likelihood of firing, yet the exact time at which the spike event occurs is determined by the network itself instead of the stimulation, allowing for more natural spike timing. Overall, the collection described below represents an extensive toolbox of engineered control tools, providing many different stimulation options.

## Control tools

### Activation

Opsins with an excitatory effect often exist in the form of a cation channel, allowing the flow of ions through the membrane against their concentration gradient. For channelrhodopsin-1 (VChR1 – (Zhang, et al., 2008)) yellow or red light will open the light-sensitive channels, allowing Na+, H+, and Ca2+ to flow into the cell. The same effect occurs with blue or green light with channelrhodopsin-2 (ChR2 – (Boyden, Zhang, Bamberg, Nagel, & Diesseroth, 2005)). Due to its success many channelrhodopsin mutants have been created to achieve various effects. All variations react fast to light exposure, allowing stimulation at millisecond precision but the kinetics of channel closure after illumination varies. ChR2, for instance, turns off around 10ms after illumination (Boyden, Zhang, Bamberg, Nagel, & Diesseroth, 2005), (Nagel, et al., 2003) while ChR2(T159C) takes 26ms to deactivate (Berndt, et al., 2011). ChETA’s are engineered to close much faster offering better temporal precision and allowing stimulation at much higher frequencies. Available ChETA’s include: ChR2(E123A) ( (Gunaydin, Yizhar, Berndt, Sohal, Diesseroth, & Hegemann, 2010)) ,ChR2(E123T), and ChR2(E123T/T159C) (Berndt, et al., 2011) which turn off after 4ms, 4.4ms, and 8ms respectively. These opsins are less suited for long pulses of light however, as the rapid closure of the channels reduces the effective light sensitivity of the cells (Gunaydin, Yizhar, Berndt, Sohal, Diesseroth, & Hegemann, 2010). Moreover channelrhodopsins tend to have a relatively low single channel conductance, and this holds true especially for the ChETA variants. Channel conductance has been improved significantly in other mutations introduced into ChR2 such as ChR2(H134R; (Nagel, Brauner, Liewald, Adeishvili, Bamberg, & Gotschalk, 2005); (Gradinaru, et al., 2007)),ChR2(T159C; (Berndt, et al., 2011)and ChR2(L132C; (Kleinlogel, et al., 2011)). However the increased amplitude of the currents comes at the expense of a much slower channel closure (18ms, 26ms, and 16ms respectively), making these mutants more suitable for driving low frequency spike trains. Both high amplitudes and faster channel closure (~10ms and 4-5ms) have been attained with the modifications ChIEF ( (Lin, Lin, Steinbach, & Tsien, 2009)) and ChRGR (Wang, et al., 2009). All of the above opsins originate from the algea Chlamydomonas reinhardtii (ChR2) and respond to blue/green light (maximally activated/sensitive to wavelengths of around 470nm). In contrast, Channelrhodopsin-1 from Volvox Carteri (VChR1; (Zhang, et al., 2008) remains responsive to wavelengths of 589nm, meaning yellow/red light can be used activate cells that express this opsin. The fact that this wavelength has no effect at all on ChR2 channels expands the prospect of cell-type specificity. For instance, expressing both ChR2 and VChR1 in different cell assemblies in the same animal allows even more finely tuned excitation. Photocurrents are much stronger in VChR1 than in ChR2, however once again these stronger currents are accompanied by longer off kinetics as VChR1 channels take up to 133ms to close. These extended channel closure times can also be seen as an advantage as cells can remain activated for as long as ~30 mins after light stimulation ( (Yizhar, Fenno, Zhang, Hegemann, & Diesseroth, 2011)). This could potentially allow the researcher to, for instance, insert an optic fiber into the brain, stimulate a certain region, remove the fiber, and observe the effect of the stimulation on the behavior of the freely moving animal for the duration of the experiment. Step-function opsins (SFO) are specially engineered to have long off-kinetics. Moreover channel closure can be induced by a pulse of yellow light (560-590nm (Berndt, Yizhar, Gunaydin, Hegemann, & Deisseroth, 2009)). This facilitates step-like control of cells, gradually bringing them closer to their firing threshold. The advantage of priming cells in this way is that it encourages a more natural firing pattern, instead of forcibly applying a certain predetermined spike train upon the cells that disregards the current state of the network. This property makes the SFO’s suitable to activate large brain regions without interfering with the specific spatial and temporal firing patterns of the microcircuits within such a region.

### Silencing

Inhibition can be realized by two different mechanisms. Either through chloride influx or proton efflux.

Halorhodopsin (NpHR) is a pump that produces chloride influx when exposed to yellow light. (Yizhar et al, 2011). Halorhodopsins tend to be expressed far less efficiently in cells than channelrhodopsins, and therefore will often need to be boosted with additional trafficking sequences to gain better expression. Currently the most stable version is eNpHR3.0 (Gradinaru, 2008, 2010) has been applied successfully in vivo (Tye et al 2011, Witten et al 2010). Both NpHR and ChR2 can be expressed in the same cell since the light sensitivities of these two opsins are sufficiently separate, allowing the researcher to control both the activation and silencing of this cell.

Proton pumps remove protons from the cell thereby hyperpolarizing the membrane. Arc (Chow, et al., 2010), or archaerhodopsin-3, from halobacterium sodomense is such a proton pump, and is activated when illuminated with green or yellow light, and reaches its peak activation at 566nm. Arc has been shown to achieve complete silencing of the cells in which it is expressed in awake, behaving mice (Chow, et al., 2010). Arc-T is a mutant of Arc that is 3.5 times more light sensitive than arc (Han, et al., 2011). Mac is a third type of proton pump, however this pump is activated by blue light, allowing the simultaneous expression of both Mac and Arc in different cell assemblies for instance, allowing separate inhibitory control of both populations. In fact a single cell could express both Mac and Arc and participate in both separately controlled assemblies. Finally another proton pump can be created through the expression of bacteriorhodopsin (eBR; (Gradinaru, et al., 2010)), which is most sensitive to wavelengths of 540nm and has a channel closure/inactivation time of 19ms.

In contrast to channelrhodopsins, which can be driven by pulses of light, these inhibitory pumps need continuous light in order to complete their photocycle, as well as avoid rebound excitation (Fenno, Yizhar, & Diesseroth, 2011). However, the application of continuous light may have several effects. Firstly, care must be taken to avoid overheating with this prolonged light exposure. Moreover, despite the presence of continuous light the slow decay of the activation of the cells due to desensitization over time must be taken into account (Nagel, et al., 2003). Curiously however, prolonged stimulation of halorhodopsin has recently been shown to result in a sustained change in the GABAergic reversal potential immediately following the light exposure, leading to an increase in the probability of synaptically evoked spiking after stimulation. The strength of NpHR photocurrents was strongly correlated with the change in the reversal potential, where stronger light stimulation led to a more pronounced change and to a subsequent increase in evoked spiking occurrences (Raimondo, Kay, Ellender, & Akerman, 2012). Finding and applying the minimum amount of light necessary to achieve the desired result can help reduce these effects of prolonged light exposure.

As revealed above optogenetic tools offer many options of possible control. Inhibition can be achieved through opsins with pumping actions such as halorhodopsin or proton pumps, while excitation can be realized through opsins such as channelrhodopsin and it’s many variations. These engineered variations increase the stimulation options significantly. Some are designed to have fast channel closure times, which is beneficial for experiments needing high frequency stimulation. Whereas slower channel closure times can be used to implement completely different dynamics, by, for instance, allowing the network to determine the precise temporal occurrence of the actual firing event by using the stimulation to bring the cell closer to its firing threshold without actually crossing it.

### Overview of opsins

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Opsin | Peak activation  | Off-kinetics | Possible stim.freq | Reference |
| Fast activation |  |  |  |  |
| ChR2(H134R) | 450 nm | 19ms | up to 20 Hz | Nagel et al. 2005Gradinaru et al. 2007 |
| ChIEF | 450 nm | 10ms | up to 25 Hz | Lin et al. 2009 |
| ChR2 | 470 nm | 10ms | up to 20 Hz | Nagel et al.Boyden et al.  |
| ChR2(T159C) | 470 nm | 26ms | slow |  |
| CatCH(L32C) | 474 nm | 16ms | up to 50 Hz | Kleinlogel et al., 2011 |
| ChETA(E123T) | 500 nm | 5ms | up to 200 Hz | Gunaydin et al. 2010 |
| ChRGR | 505 nm | 8-10ms | slow | Wang et al, 2009 |
| E123T+T159C | 505 nm | 8ms | up to 40 Hz | Berndt et al, 2011 |
| C1V1 | 540 nm | 156ms | slow | Yizhar et al, 2011 |
| VChR1 | 545 nm | 133ms | slow | Zhang et al, 2008 |
| Slow Activation |  |  |  |  |
| ChR2 C128S | On:470, Off:~570 | 1.7 min | N/A | Berndt et al. 2008 |
| C128S + D156A | On: 445, Off: 590 | 29 min | N/A | Yizhar et al. 2011 |
|  |  |  |  |  |
| Inhibition |  |  |  |  |
| NpHR | 589 ms | 41ms | continuous | Zhang et al. 2007 |
| eNpHR | 590 ms | 4.2ms | continuous | Gradinaru et al. 2010 |
| Arch | 566 ms | 19ms | continuous | Chow et al. 2010 |
| Arch-T | 566 ms | 15ms | continuous | Han et al. 2011 |
| eBR | 540 ms | 19 ms |  | Gradinaru et al 2010 |
|  |  |  |  |  |

Table 1: Overview of different opsins available and their characteristics (peak activation wavelength, off-kinetics, and possible stimulation frequencies).

## Gene Delivery

### Viral

A lentivirus (LV) or adeno-associated virus (AAV) can be used to deliver the transgenes for optical expression in a wide variety of animals. The injected virus inserts multiple copies of a gene into each target cell, resulting in high levels of gene expression that can be sustained for several months. After injection the virus will need 2 (LV) to 3 (AAV) weeks before functional levels of gene expression are reached (Zhang, et al., 2010). This method is less suited when high cell specificity is required, due to the fact that only short promoters are able to fit into a viral vector (Carter & de Lecea, 2011). Also, when local expression is desired LV is a better choice, considering expression achieved with AAV tends to be more diffuse.

### Transgenic animals

While viruses are limited to short promoter sequences, long promoter sequences are possible in transgenic mice, enabling a high level of cell-type specificity. However several months need to be invested in order to establish a reliable mouse line. Generally at least 6 generations are required before a stable expression is reached. The costs, time, and effort invested in setting up and maintaining a transgenic mouse line are offset by the benefits such as the control over the exact genetic makeup of the animals raised in the same environment, reducing variability over subjects, and the convenience of having animals on hand when needed (Yizhar, Fenno, Zhang, Hegemann, & Diesseroth, 2011). The main issue with transgenic animals concerns the low levels of expression, most likely due to the reduced gene copy number per cell (Dugué, Akemann, & Knöpfel, 2012)

Shortcomings of both these methods can be compensated through their combination. Using viruses in transgenic animals results in better cell-type specificity and stronger expression (Fenno, Yizhar, & Diesseroth, 2011).

### In utero electroporation

Another method allowing the insertion of long promoter sequences, and thus capable of high cell-specificity is in utero electroporation. Cells can be targeted before the birth of the animal. The time of development at which the DNA is inserted will determine the cortical layer affected by the opsin expression, which is why this technique is also referred to as ‘spatiotemporal targeting’ (Gradinaru, et al., 2007). Another major advantage of this technique is that many copies of the same gene can be introduced into every targeted cell, ensuring good expression (Zhang, et al., 2010).

Table 2: overview of gene delivery methods

|  |  |  |  |
| --- | --- | --- | --- |
|  | Viruses | Transgenic animals | In utero electroporation |
| expression levels | High | Low | High |
| cell-type specificity | Low | High | High |
| investment | Low | High | Low |

##

## Light Delivery

The approach to stimulation depends on the goal. Light can be delivered with rectangular pulses of varying durations, but it is also possible to stimulate with various waveforms. For instance a waveform can be used to simulate the naturally graded position-related spiking of hippocampal neurons during exploration (Stark, Koos, & Buzsáki, 2012)

Light can be delivered to the target area though very thin and flexible cables called fiberoptic cables. These fibers can be inserted into the brain in various ways. In acute experiments fibers may be placed into the brain directly.

Chronic recordings require more permanent solutions. One option is to insert a cannula as a guide along which a probe can be inserted, ensuring it enters the same region every time. In the case of viral vector delivery the cannula serves as a useful aid in delivering the virus. This also ensures that the fiber, later inserted through the same cannula, will be directed to the area affected by the opsin. Reinserting a fiber for every experiment has several drawbacks however. First of all, tissue damage will result from the repeated fiber insertion. Also, frequent fiber breakage is common. Additionally, an increased risk of infection exists, due to the exposure of the brain to the environment through the cannula (Zhang, et al., 2010).

Alternatively, a piece of fiber can be chronically implanted that reaches from the target area within the brain to just above the skull, where it can be coupled to a light source.

### Lasers

Diode Pumped Solid State (DPSS) Lasers

Lasers are a commonly used light source in optogenetics due to their high power capabilities. This high power can facilitate beam splitting, wavelength division, and can compensate for poor coupling. When using lasers always be aware that the actual emitted light will be only a fraction of the maximal output power of the laser. Furthermore the shape of the pulse may have unexpected temporal characteristics (Yizhar, Fenno, Zhang, Hegemann, & Diesseroth, 2011)

Light flow from the laser into the optical fibers can be achieved in several ways. Couplers can be used to attach the laser head to the end of the fiber. However, up to 50% of light may be lost through this connector, therefore more power will be needed for the desired amount of light to reach the target area. Alternatively a lens can be used to focus the laser beam onto the fiber. The mirrors can be used to direct the emitted light into the fiber (Yizhar, Fenno, Zhang, Hegemann, & Diesseroth, 2011). Attaching the laser to an aluminum breadboard, or another large heat sink, prevents overheating (openoptogenetics.org).

Supplying diode pumped light to multiple sources for the delivery of several different wavelengths to activate various opsins within different regions would require multiple DPSS lasers. Furthermore, the animal would need to be attached to multiple optic fibers, increasing the chances of fiber breakage and limiting the free movement of the animal. For cases such as these, laser-diodes would be better suited. At barely half a millimeter these diodes are extremely light weight and can be coupled to fibers, which, in turn, are attached to individual shanks of silicon probes or tetrodes (Stark, Koos, & Buzsáki, 2012) allowing multiple areas to be precisely targeted with a unique pattern or wavelength of stimulation.

### LED

For superficial stimulation LEDs can be positioned directly above the brain and illuminate cortical layers through a cranial glass window. LEDs are small yet powerful, thus will not impede free movement of the animal while having enough intensity to reach all cortical layers (Carter & de Lecea, 2011)

LEDs can also be coupled to optical fibers to target deeper area’s. However, due to the highly divergent beam pattern from LEDs the coupling efficiency is generally poor. The desired light intensity can still be achieved by using a more powerful LED however ( (Gradinaru, et al., 2007), (Petreanu, Huber, Sobczyk, & Svoboda, 2007)). The use of LEDs is not yet widespread in the optogenetics community primarily due to this coupling difficulty. It is worth noting the potential of LEDs however for optogenetic experiments. They are lightweight, thus well suited for behavioral experiments. Moreover they are less expensive, easier to control, and more reliable than lasers (Grossman, et al., 2010).

### Other light sources

In principle any light source could be used, including incandescent lamps or arc lamps. These will need to be equipped with shutters and filters to modulate light output to the desired amount and frequency. These light sources can also be acquired pre-built, complete with shutters and filters, and can reach pulse frequencies of up to 500Hz (Boyden, 2005). The use of shutters, however, entails that only on/off light pulses can be delivered. In contrast, lasers and LEDs are capable of delivering a graded modulation of intensity (Yizhar, Fenno, Zhang, Hegemann, & Diesseroth, 2011)

### Combining stimulation and recording

Neurons must be no more than 60 micrometers from the electrode for their activity to be detected. Therefore, stimulating neurons and observing the response requires stimulating close to the electrode. When using an optogenetic stimulation technique a light guide must therefore be located close to the electrode (Stark, Koos, & Buzsáki, 2012) Royer and colleagues (2010) have approached this challenge by attaching optic fibers to the shanks of a silicon probe, allowing each shank to be activated individually. This also opens up the possibility to deliver light of different wavelengths to different probes simultaneously. The DPSS lasers used by Royer connect with bulky fibers to the headstage of the animal. This approach would require multiple laser and thus multiple fibers, restricting the free movement of the animal.

In a recent publication Stark and colleagues (2012) describe the coupling of lightweight laser or LED diodes to optic fibers. This construction was then coupled to silicon probes or wire tetrodes. Using individual diodes in this manner has many advantages. Firstly, it eliminates the need for the animal to be tethered to optic fibers in order for light to be delivered considering in the diodes are light and small enough to be located on the head of the animal. Moreover, the separate control over every diode allows extremely precise spatial and temporal stimulation patterns to be applied.

# Closing the loop: conclusion

The similarity between the orderly, linear control systems from the field engineering and the highly chaotic, nonlinear biological feedback systems may not be apparent at first. The “well-behaved”, carefully crafted control systems such as the air conditioner seem far removed from neural dynamics. First of all, most engineered systems are designed with a single task in mind, such as maintaining a certain desired state. The goal of the control system is often to optimize the manner in which this task is performed.

An in vivo, closed-loop approach is not yet common practice in the field of neuroscience. One exception can be found in the design of brain machine interfaces.

In the case of a BMI precise control is desired over the control system consisting of a neural system and an artificial system that interfaces with it. Not surprisingly, this is also the branch of neuroscience that currently appears to make the most use of engineering principles such as closed-loop system design (van Greven, et al., 2009).

 For research purposes however, such a goal is not necessarily applicable. For instance, a complex dynamical system that we do not fully understand can be probed, not to optimize the system in any way, but simply to observe the control law necessary to maintain a certain state in the system or to produce a particular output. The ‘control law’ of a control system designed to maintain homeostasis simply subtracts the actual output of the system from the desired output. However, when attempting to relate a particular behavior to a specific spatiotemporal neural firing pattern the desired outcome is the behavior and the control law aims to manipulate the neural system in such a way as to generate this behavior. The control law in this case would be an algorithm in the form of a parametric or nonparametric model that observes the neural system and attempts to extract the relevant pattern. When the found pattern is then acted upon the outcome of the stimulation will determine the accuracy of the algorithm, or control law. For instance, if inhibiting the cells involved in the pattern predicted by the algorithm leads to the suppression of the behavior we could conclude that the discovered spatiotemporal pattern of activity is necessary in coding for the behavior. If excitation of this same pattern results in the generation of the behavior it is possible to conclude that this pattern of activity is sufficient to produce the studied behavior. In this manner the same control engineering framework can be implemented to achieve a different goal, namely to improve our understanding of the mechanisms underlying the functionality of a complex system.

The control-engineering framework has proven to be flexible enough to provide a useful structure both for experimental neuroscience as well as clinical applications such as brain machine interfaces. Furthermore, the inclusion of theoretical concepts such as those from control theory into an experimental context will undoubtedly lead to fruitful interactions between theoretical and experimental neuroscientists, benefitting both fields by verifying theoretical concepts in experimental contexts and vice versa by providing a language to translate qualitative ideas regarding neural dynamics into quantitative hypotheses that can more accurately describe the precise dynamics to be verified empirically.

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