Drumming with dopamine neurons

Resonance and synchronization in the Ventral Tegmental Area

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CHAPTER 1

General introduction
1.0 Ventral Tegmental Area

The ventral tegmental area (VTA) is the subject of diverse neuro-scientific research: from pharmacological and cognitive to cellular and biochemical. The VTA's dopamine projections reach extensive brain areas, such as the prefrontal cortex and nucleus accumbens. Among others the VTA plays a role in memory (Fujisawa and Buzsáki, 2011), learning (Kim et al., 2012), reward prediction (Schultz, 1997) and the reward system. The detailed functionality of the local VTA network and its role in cognitive processing is largely unknown and in vivo studies do not allow the study of this local network in isolation. The purpose of this thesis is to study the local VTA network and to better understand how it supports the varied tasks it is implicated in. In this thesis we study the lateral VTA, which contains mesolimbic projecting dopamine neurons with conventional phenotypes (Björklund and Dunnett, 2007; Lammel et al., 2008). These conventional dopamine neurons exhibit spontaneous low frequency spike activity (in vivo and ex vivo) (Bayer et al., 2007; Bowery et al., 1994; Grace and Onn, 1989; Werkman et al., 2001). Most research of the VTA's neuronal activity revolves around single electrode recordings (ex vivo) (Hand et al., 1987; Werkman et al., 2001) or tetrode recordings (in vivo) (Fujisawa and Buzsáki, 2011). In our research we used a Multi-Electrode-Array (MEA) (Taketani and Baudry, 2010) to record the spike activity from multiple spatially separated dopamine neurons simultaneously. This allowed us to study the activity of the lateral VTA on the level of the micro-circuit with statistic measures aimed at understanding network interactions.

1.0.1 Location of the VTA

The VTA consists of a few heterogeneous groups of neurons lying together close to the midline on the floor of the midbrain (mesencephalon) (Oades and Halliday, 1987). A short description of the major nuclei bordering the VTA, shows the position of the VTA more precisely. Rostrally extend the mammillary bodies and the posterior hypothalamus (diencephalon). The oculomotor fibers are located dorsolateral to the VTA. Dorsally and partly through the VTA fibers pass from several brainstem nuclei. Particularly the Raphe nuclei extend dorsally from the caudal border. Caudally to the VTA lies the pons and hindbrain (rhombencephalon). The VTA is located bilaterally on the midline with a roughly semicircular appearance in coronal section (Fig. 1.1). Lateral to the VTA is the substantia nigra (SN) (Fig. 1.1). It has been difficult to make a clear anatomical segregation between these two midbrain dopamine neuron groups. In fact, the VTA and SN pars compacta (SNC) have been considered as a continuum on the basis of the close similarities of their internal cellular organization and neurotransmitter systems (Takada and Hattori, 1987). 3D reconstructions of stacks of Nissl stained brain slices revealed the boundaries and volumes of the VTA and SN (Domesick, 1988). The VTA and SN compacta were shown to be anatomically continuous in this reconstruction (Domesick, 1988). However, by retrograde tracing of axonal projections the VTA can be differentiated from the SN (Takada and Hattori, 1987) (see further section 1.0.2).
1.0.2 Connections of the VTA with other brain regions

Five systems of efferent fibers involving the VTA are distinguished: mesostriatal, mesolimbic, mesocortical, mesodiencephalic, mesorhombencephalic (Oades and Halliday, 1987; Swanson, 1982). The mesostriatal pathway refers to the VTA projection to the anteromedial and ventral neostriatum. The mesolimbic pathway targets the nucleus accumbens (NAc), amygdala, the supragenual, cingulate cortex and the hippocampal complex (a subset of these connections are shown in Fig. 1.2). In return the hippocampus exerts influence over VTA dopamine neuron activity through the NAc (Floresco et al., 2001) and the VTA receives extensive inputs from the amygdala (Watabe-Uchida et al., 2012). The mesocortical pathway refers to the projections to the neocortices including projections to sensory, motor and association areas (Fig. 1.2). The projection to the prefrontal cortex (PFC) is reciprocal (Carr and Sesack, 2000; Oades and Halliday, 1987). Importantly, VTA dopamine neurons and GABAergic neurons (see 1.1.3) project to the prefrontal cortex allowing the projection to have inhibitory and excitatory effects (dopamine D₁-receptor) on the PFC (Carr and Sesack, 2000). The mesorhombencephalic pathway include the projections to the major monoaminergic nuclei, with the SN as its main recipient. Other targets include the reticular formation, Raphe nuclei and the locus coeruleus. Lastly, the mesodiencephalic pathway includes connections to the posterior and medial hypothalamus (Oades and Halliday, 1987). In general, the dopaminergic projections originating from the VTA innervate two major target areas, the mesocortical (PFC) and mesolimbic (NAc) structures (Fig. 1.2), whereas the SN is the source of the mesostriatal pathway (Oades and Halliday, 1987) (nigrostriatal, Fig. 1.2). The mesodiencephalic and mesorhombencephalic projections originate from both VTA and SN.
1.0.3 VTA neuron types and connections

The VTA consists of dopaminergic (~70 %), GABAergic (~30 %) and glutamatergic neurons (~2 %), which are spatially mixed (Nair-Roberts et al., 2008; Oades and Halliday, 1987; Omelchenko and Sesack, 2009; Swanson, 1982). The VTA dopamine neurons have been subdivided on the basis of their projection targets and electrophysiological properties. A clear functional distinction was found between non-conventional VTA dopamine neurons projecting to PFC and NAc core compared to those projecting to NAc lateral shell that display the conventional dopamine neuron electrophysiological characteristics (Lammel et al., 2008). The conventional dopamine neurons fired at lower frequencies and had larger after-hyperpolarizations (Lammel et al., 2008). In our experiments we recorded from the lateral VTA, which contains a homogenous population of conventional dopamine neurons (Lammel et al., 2008; Roeper, 2013). The GABAergic neurons exhibit rapid non-bursting firing, typical of interneurons (Steffensen et al., 1998). Besides being subject to GABA inhibition, they have NMDA receptors (Steffensen et al., 1998). Glutamate neurons within the VTA are a more recent discovery and they make up around 2 % of the population and they synaptically connect with local dopaminergic and non-dopaminergic neurons (Dobi et al., 2010; Morales and Root, 2014; Omelchenko and Sesack, 2009). Local synaptic connectivity also arises from GABAergic neurons projecting onto the dopamine neurons and between the GABAergic neurons themselves (Omelchenko and Sesack, 2009). Gap junctions between soma and dendrites of VTA dopamine neurons have also been reported (Bayer and Pickel, 1990). Besides these anatomical synaptic connections, there are other means by which the VTA dopamine neurons might interact, namely through somadendritic release of dopamine (see 1.2.2).
1.0.4 VTA pharmacology

The dopaminergic system is one of the major neurotransmitter systems in the brain, which include the glutamatergic, GABAergic, noradrenergic, serotonergic and cholinergic systems. A unique property of a number of these neurotransmitters systems (like noradrenergic and serotonergic, but not glutamatergic and GABAergic) is that their signals originate from 'hubs' in the brainstem. For the dopaminergic system these are the VTA and SN, which send projections to large parts of the sub- and neocortex (Oades and Halliday, 1987; Swanson, 1982; Arias-Carrión et al., 2010). Given that the dopaminergic signals have such a spatially extended influence on brain activity, one would suspect that they carry an important message (e.g. reward, Arias-Carrión et al. (2010)), but also that they may play a central role in certain pathological conditions (e.g. addiction, Bonci et al. (2003)). Pharmacological research within the VTA has focused on among others antipsychotics (Westerink, 2002) and addiction (Bonci et al., 2003). Disturbances of the mesolimbic and mesocortical dopaminergic pathways that originate from the VTA have been associated with multiple psychiatric disorders including ADHD, addiction and schizophrenia (Roth et al., 2003; Thomas et al., 2008; Viggiano et al., 2002). The VTA dopamine neurons with their spontaneous activity are an ideal target for detailed pharmacological interrogation, mainly performed on ex-vivo midbrain slice preparations. The main targets of research have been the dopamine D2-autoreceptors, which regulate the activity of dopamine neurons and control the release, uptake and synthesis of dopamine (Ford, 2014). These receptors activate inhibitory G-protein-activated inwardly rectifying potassium channels (GIRK). Through these ion-channels the D2-receptors, found at axonal and somadendritic sites, influence the firing patterns of dopamine neurons and thus the release of dopamine at terminals in target regions (Ford, 2014). Interestingly, non-conventional ventromedial VTA dopamine neurons projecting to the PFC express less D2-receptors and GIRK channels than conventional dopamine neurons and exhibit no functional response to D2-receptor activation (Lammel et al., 2008; Roeper, 2013). Classical experiments with typical and atypical antipsychotics, which are D2-receptor antagonists, showed elevated firing rates of the VTA dopamine neurons during administration (Bowery et al., 1994; Hand et al., 1987; Werkman et al., 2001). For a description of the spontaneous activity with low firing rates see section 1.1.1. The dopamine D2-receptors, which the antipsychotics mainly act on, have similar properties in VTA and SN dopamine neurons, making these areas difficult to distinguish based on actions of selective ligands interacting with these receptors (Werkman et al., 2001). GABAergic neurons make up about 30% of the VTA neurons and besides projecting to various areas, such as the PFC (Carr and Sesack, 2000), they make local synaptic connections with dopamine and other GABAergic neurons. The GABA agonist baclofen reduced VTA dopamine neuron firing rates and increased regularity of firing, measured as a decrease in burst firing. This effect of baclofen was mediated by GABA_{b} receptors linked to potassium channels on the dopamine neurons (Erhardt et al., 2002; Seutin et al., 1994).
1.0.5 Function of the VTA and its relation to other areas

In vivo studies using optogenetic stimulation of VTA dopamine neurons indicate a causal role of the VTA in modulating social behavior (Gunaydin et al., 2014) and reward-aversion processing (Ilango et al., 2014) in mice. For instance, it was shown that inhibitory optogenetic stimulation of the dopamine neurons in the VTA had a negative effect on social interactions with other animals, whereas excitatory stimulation increased the total time of social interactions (Gunaydin et al., 2014). Interestingly, the effect was specific to the VTA dopamine neurons projecting to the Nac (Gunaydin et al., 2014). The role of the VTA in affective functions has been studied extensively. The VTA plays a major role in reward-prediction (Schultz, 1997) and handling novel information (Lisman and Grace, 2005). The dopamine system is often seen as synonymous to the reward system. In this context, VTA dopamine neurons encode the prediction of rewarding events in behavioral tasks. The output of the dopamine neurons is a prediction error signal, thus coding the difference between prediction and actual outcome (Montague et al., 2004; Redgrave and Gurney, 2006; Schultz, 1997, 1998; Cohen et al., 2012). This error signal could be used by downstream areas to enable learning from experience. Learning and thus handling novel information has been attributed to a loop between the VTA, hippocampus and the NAc (Lisman and Grace, 2005; Lisman et al., 2010). Here, activation of the loop is initiated when the hippocampus detects newly arrived information that is not already stored in long-term memory. The resulting novelty signal is conveyed via the NAc to the VTA where it contributes to its novelty-dependent activity. More recent work implicates the VTA directly with behavioral learning tasks. Synchrony between the activity of VTA dopamine neurons increased during conditional learning tasks in rats (Kim et al., 2012). In section 1.0.2 the anatomical pathways between the VTA and other areas are described. The mesolimbic and mesocortical pathways are combined in a PFC-VTA-hippocampus axis proposed by Fujisawa and Buzsáki (2011). They found cross-area synchrony of neuronal activity between the PFC, VTA and hippocampus. A 4 Hz rhythm is dominant in the PFC and VTA, modulating both local gamma oscillations and neuronal activity, whereas synchrony of neuronal spikes in the hippocampus is mainly under control of theta oscillations. Fujisawa and Buzsáki (2011) hypothesized that phase coupling between the 4 Hz and theta oscillators, and their combined modulation of local gamma oscillations, may be a mechanism for connecting the entorhinal-hippocampal system with the mesolimbic dopaminergic reward system.
1.1 Brain rhythms and oscillations

Section 1.0 discussed the anatomical properties of the VTA and the behavioral functions it is implied in. The way that VTA neurons encode information likely depends strongly on interactions among these neurons, which can be critically shaped by and give rise to neuronal oscillations. In this thesis, neuronal oscillations will be a central topic of investigation, and this is motivated by two key pieces of evidence: 1) The finding that VTA neurons show strongly rhythmic firing, which can be sustained spontaneously both under ex vivo and in vivo conditions (Koyama et al., 2005; Khaliq and Bean, 2010; Paladini and Roeper, 2014) and 2) The finding that VTA neurons, in awake animals, have very strong synchronization around these frequencies with other connected brain areas like the PFC and hippocampus (Fujisawa and Buzsáki, 2011). The way in which VTA dopamine neurons synchronize with each others’ rhythmic firing could shape the interactions with other brain regions. In this section we give a brief overview of research on oscillations and synchrony in the neurosciences with a focus on the VTA and its neuronal oscillators.

1.1.1 VTA rhythms

Midbrain dopamine neurons have very typical rhythmic activity patterns at relatively low frequencies (1-10 Hz, in vivo). This rhythm is intrinsic as isolated VTA dopamine neurons show regular pacemaking spike activity in cultures (Koyama et al., 2005). Although pacemaker activity is characteristic for midbrain dopamine neurons, the mechanisms differ between the SN and VTA. The mechanisms underlying the pacemaking activity in dopamine neurons have been studied with patch clamp recording methods. In the VTA the experimental results point at a persistent sodium current (Khaliq and Bean, 2010), while in the SN the rhythm is driven by slow oscillating calcium concentrations (Drion et al., 2011; Putzier et al., 2009). The lateral VTA we record from during our study contains mesolimbic projecting conventional dopamine neurons which fire at low frequencies (1-4 Hz, ex vivo, (Lammel et al., 2008)).

One of the main question posed in this thesis is: “Can the pacemaker like activity of the individual dopamine neurons contribute to oscillations in the output of the VTA?” In vivo studies have described 4 Hz synchronized oscillations in the VTA (Fujisawa and Buzsáki, 2011). This synchronized network activity of the VTA could be either driven through local connections among VTA neurons, or it might alternatively be driven by top-down inputs. The various possible underlying mechanisms are our next topic of discussion.

1.1.2 Network oscillations

Mechanisms

When we consider the synchrony on larger scales within the brain, that of a whole brain area or between brain areas there are a few distinct mechanisms that can be at play. A heterogeneous population of principal neurons and interneurons can generate their own oscillation in their output by local excitatory and inhibitory interactions (Wang, 2010). Three basic mechanisms exist this: recurrent excitation between principal neurons, mutual inhibition between interneurons, and feedback inhibition through the excitatory-inhibitory loop (Wang, 2010). As we have seen in section 1.0.3 all the connections to support such interactions
exist within the VTA, namely principal-principal, GABAergic-GABAergic, and GABAergic-
principal connections with the addition of the connections made by glutamatergic neurons. The neuron population can also interact with the rhythm received from its input areas, by following it or modifying it. A basic concept is that of the pacemaker, which is a brain area that drives the rhythm of its downstream projection areas, by entraining them to its own rhythm. There can be more than one pacemaker in a system, entraining each other through bi-directional connections (Buzsaki, 2009).

Function of rhythms

Although, the brain exhibits rhythms at many different scales and contexts, there is no consensus about their importance in the functioning of the mammalian brain (Sejnowski, 2006). Brain oscillations are suggested to facilitate the representation of sensory information, being the temporal reference structure relative to which spike times become meaningful (Sejnowski, 2006). It is also thought that, rather than representing information, oscillations and synchrony regulate the flow of information in neural networks, as described by Communication-Through-Coherence (CTC) (Fries, 2005, 2015; Sejnowski, 2006). CTC posits that brain oscillations orchestrate the communication between brain areas through mutual entrainment. A third non-exclusive possibility is that oscillations assist in the storage and retrieval of information in neural circuits, specifically network oscillations are thought to organize presynaptic and postsynaptic spike times, thus improving spike-timing-dependent plasticity (Sejnowski, 2006).

Network oscillations in the VTA

As discussed in Section 1.0.5, the spike output of the dopamine neurons can act as a prediction error signal, encoding the difference between a predicted reward and the actual outcome (Montague et al., 2004; Schultz, 1997, 1998). This error signal can enable downstream areas to learn from experience. Learning and thus handling novel information has been attributed to a loop between the VTA, hippocampus and the NAc (Lisman and Grace, 2005; Lisman et al., 2010). Here, activation of the loop is initiated when the hippocampus detects newly arrived information that is not already stored in long-term memory. The resulting novelty signal is conveyed via the NAc to the VTA where it contributes to its novelty-dependent activity. More recent work implicates the VTA directly with behavioral learning tasks. Coherence between the rhythmic activity of VTA dopamine neurons increases as rats learn a cognitive task (Kim et al., 2012). Although, the increased functional connectivity seen by Kim et al. (2012) can originate from a common upstream pacemaker, it can also point to strengthening of functional connectivity between VTA dopamine neurons through learning. Functional connectivity is measured by the coherence between the spike activity of two neurons.

Currently a pacemaker of low frequency oscillations is sought after in a set complex interaction between three brain areas, dubbed the PFC-VTA-hippocampus axis. In section 1.0.2 the anatomical pathways between the VTA and other areas are described. The mesolimbic and mesocortical pathways are combined in a PFC-VTA-hippocampus axis proposed by Fujisawa and Buzsáki (2011). They found cross-area synchrony of neuronal activity between the PFC, VTA and hippocampus. The VTA was proposed as a candidate pacemaker for a 4 Hz rhythm (Fujisawa and Buzsáki, 2011) synchronizing these various areas. Whether the local
interactions within the VTA could lead to significant oscillations in its population output is one of the main topics of this study.

1.1.3 Resonance properties

As described in sections 1.1.1 and 1.1.2, the rhythmic dopamine neurons in the VTA together are a candidate for a pacemaker role for other brain regions. While previous work has looked at the output behavior of the VTA, the input/output characteristics of VTA neurons are not understood well. These characteristics are critical for our understanding of the VTA’s function. Key in our study are the concepts of resonance and entrainment.

Resonance is similar to entrainment discussed in section 1.1.2, but subtly different. Resonance occurs in the response of a physical system to a driving force. It typically occurs when driving the system with specific frequencies at which there is almost no energy loss from input to output. It is different from entrainment, because the physical system, that is driven, does not have its own actively maintained rhythm during resonance (Pikovsky et al., 2002). VTA dopamine neurons have an actively maintained rhythm, which we will study by driving it pulsed stimulation. Will the neuron entrain to our stimulation and what are the frequency characteristics? What happens when the dopamine neuron is driven by a noisy stimulus? Might this allow us to study its resonance characteristics?

In general, the brain is noisy, which leads to questions about the functional impact of neuronal noise (Ermentrout et al., 2008). Although, noise is most often thought of as disrupting organized activity, for instance interfering with the encoding of stimuli, recent theoretical and experimental work has shown that noise can play a constructive role (Ermentrout et al., 2008). Noisy inputs can lead to increased reliability or regularity of neuronal firing in single neurons and across populations (Ermentrout et al., 2008). The distinction between noise and signal is not clear cut and will probably change as our understanding of the brain grows. In any case, resonance to noisy inputs can be an additional function for rhythms and oscillations in the brain (Ermentrout et al., 2008; Galán et al., 2006). Here, we will study the resonance properties of the VTA dopamine neuron population to rhythmic and noisy inputs to understand the input-output properties of the VTA circuit.
1.2 Research questions and thesis outline

Our study is aimed at the transition from neuronal effects to network effects within the lateral VTA. Do VTA dopamine neurons interact locally with each other? Are these interactions altered by dopamine specific pharmacology? Can we manipulate the intrinsic rhythm and interactions of the VTA through optogenetics? In the next sections we discuss these and other questions spread over three chapters.

1.2.1 Chapter 2

As discussed in section 1.1.1 VTA dopamine neurons exhibit slow rhythmic spike activity (1-4 Hz \textit{ex vivo}, 1-10 Hz \textit{in vivo}). The VTA is a candidate for a pacemaker role in a PFC-VTA-hippocampus axis described in section 1.0.5. It is unknown whether the local neuronal interactions in the VTA can lead to oscillations in the total neuronal activity output of the VTA. Such oscillations could arise from synchronization between the spontaneous rhythmic activity of the VTA dopamine neurons. Our goal was to measure the functional connectivity between simultaneously recorded VTA dopamine neurons and assess whether the rhythms of the individual could sum to a population oscillation through entrainment. Thus, we asked whether the VTA dopamine neurons are functionally connected? The functional connectivity was assessed with the Paired Phase Consistency (PPC, (Vinck et al., 2010)) for all pairs of neurons within the simultaneously MEA recording. To study the functional connectivity more thoroughly we investigated how it depends on the rhythmicity and activity levels of the dopamine neurons. We elevated the VTA dopamine neuron activity in two ways, by increasing the extracellular potassium concentration and by administration of glutamate to the bath solution applied to the slice preparation. These two treatments both increased the firing rates of the individual dopamine neurons. Next, we studied whether they had differential effects on the rhythmicity and the functional connectivity among VTA dopamine neurons.

1.2.2 Chapter 3

How does the effect of dopamine D$_2$-receptor agonists or antagonists on individual neuronal activity translate to the level of neuron population activity? Besides the anatomical synaptic connections, as described in section 1.0.3, there is another means by which the VTA dopamine neurons communicate, namely somadendritic release of dopamine. Here, the dopamine neurons leak dopamine into the extracellular medium at their soma and dendrites during neuronal firing activity (Cragg et al., 2001). These extracellular dopamine levels follow the neuronal activity and through diffusion activate the dopamine D$_2$-receptor on nearby VTA dopamine neurons, leading to auto- and cross-inhibition. This mechanism is called volume transmission (Adell and Artigas, 2004; Cragg et al., 2001). Such interactions have been directly observed between dopamine neurons in the SN (Vandecasteele et al., 2008) and likely enrich the local interactions within the VTA. Although, the effect of dopamine on the individual dopamine neuron is well understood, its effect on a population of VTA dopamine neurons is unclear. We assessed the sensitivity to dopamine for all dopamine neurons within the recorded populations using the selective dopamine D$_2$-receptor agonist quinpirole. Are some VTA dopamine neurons more sensitive to quinpirole than others? If so, what does this mean for their interactions with the extracellular dopamine signal? Are the relatively dopamine
insensitive neurons more leading as they are less inhibited by the dopamine signal, while still inhibiting other neurons with their dopamine release? To assess the follower-leader relations we computed the Granger causality between pairs of neurons (Ding et al., 2006; Granger, 1969). To verify the effect of quinpirole on the follower-leader relations we used the selective dopamine D₂-receptor antagonist sulpiride to block the effects of quinpirole.

1.2.3 Chapter 4

The VTA is part of various pathways and the origin of two important dopaminergic pathways, one to the mesolimbic and one to the mesocortical brain structures, as described in section 1.0.2. Besides innervating other brain areas, the VTA also receives inputs from many areas (Watabe-Uchida et al., 2012). As described in section 1.1.1, the rhythmic dopamine neurons in the VTA together are a candidate for a pacemaker role for other brain regions. While previous work has looked at the output behavior of the VTA, the resonance characteristics of VTA neurons remain not described. As these characteristics determine the input-output properties of the VTA circuit, they are critical for our understanding of its function. Optogenetic stimulation is the ideal tool to very selectively manipulate the firing of individual dopamine neurons. Therefore, it can be used to determine the entrainment and resonance characteristics of the VTA dopamine neurons. In addition to rhythmic stimulation regimes, we wonder whether noise stimulation could lead to network states via noise-induced-synchrony, a recent addition to the study of brain computations (Ermentrout et al., 2008). To this end, we created F1 mice selectively expressing channelrhodopsin in the VTA dopamine neuron membranes targeting the Pitx3 locus (Madisen et al., 2012; Smidt et al., 2012). Can we entrain a VTA dopamine neurons’ activity to the regular pulses of the optogenetic laser stimulation ex vivo? If so, how does regular compare to random stimulation regime? Our experiments are analogues to the modeling studies by (Hata et al., 2010) with the goal to understand the difference between entrainment and noise-induced-resonance (Ermentrout et al., 2008) in the local VTA network.
1.3 Techniques

1.3.1 Multi-electrode-array recordings

Previously Multi-Electrode-Arrays (MEAs, see box on page 14) with planar electrodes were used to simultaneously record dopamine neurons in the SNc (Berretta et al., 2010). We used the 60 electrode 8-by-8 3D MEA to record from populations of spontaneously active dopamine neurons (8 to 35 neurons) in the lateral VTA simultaneously. The lateral VTA is roughly circular in coronal slices and a perfect fit for the standard 8-by-8 electrode layout, increasing the yield of the recordings. Long-term experiments were performed in combination with substance administration in the bath solution or optogenetic stimulation. The long-term simultaneous recording of the stable activity of a population of VTA dopamine allowed us to probe their local interactions. The distance between electrodes (100 \( \mu \text{m} \)) is small enough to make synaptic interactions viable and to make recording the same neuron on two electrodes highly unlikely. The extracellular action potentials (spikes) were detected in the MEA signals and individual neurons were identified and their spike timestamps were used for further analysis.

The MEA has seen development since the 1950s, mainly for implants. The first experiments with cultured cells on an array of planar electrodes were done in 1972. Before the 1990s MEAs were cost prohibitive, but recently computing power and commercial MEA systems have led to an increase in their use in physiological experiments. The most used setup is 60 micro-electrodes each spaced 100 \( \mu \text{m} \) with a diameter of 30 \( \mu \text{m} \) in an 8-by-8 configuration. To improve the signal-to-noise ratio when performing experiments on \textit{ex vivo} brain slices, 3D MEAs were developed with protruding electrodes which can penetrate the dead layer of cells caused by slicing (Olivier et al., 2002). These 3D MEAs allow recording of extracellular action potentials from individual neurons and the 100 \( \mu \text{m} \) separation of the electrodes ensures the recording of separate neurons, although a single electrode can pick up more than one neuron.

1.3.2 Optogenetic stimulation for probing resonance characteristics

Optogenetics (see box on page 15) has been used successfully to control VTA dopamine neuron activity \textit{in vivo} (Adamantidis et al., 2011; Tsai et al., 2009), expressing the protein with a viral vector. Optogenetics allowed them to probe the role of the VTA dopamine neuron activity during reward-seeking and behavioral flexibility. Cre recombinase was expressed in the VTA dopamine neurons by binding it to the tyrosine hydroxylase (Th-Cre knock in mice) an enzyme important in the production of the dopamine precursor L-DOPA. In our work we targeted Cre expression via the Pitx3 locus, a homeobox transcription factor well known for its involvement in midbrain dopamine neuron development, using the Pitx3-Cre knock in mice (Luk et al., 2013; Smidt et al., 2012). These mice were crossed with a homozygous Lox-ChR2-YFP mouse (Madisen et al., 2012) allowing reproducible optogenetic expression across brain slice experiments. Optogenetic stimulation of the full recorded population was
enabled using wide-field laser stimulation. This allowed precise temporal stimulation of the entire recorded population of VTA dopamine neurons, driving them with a common pulse stimulus. Short pulses were administered in regular and random (Poisson) regimes to study the entrainment and resonance characteristics of the VTA, which are largely unknown.

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**Optogenetics**

Optogenetics have sparked a new field in neuroscience allowing stimulation of specific target neurons *ex vivo* and *in vivo* with a high temporal resolution. Optogenetic tools have been developed since the 1970s but were recently introduced in neuroscience allowing precise causal control of neuronal activity of intact systems (Deisseroth, 2010). The light-activated channelrhodopsin ion-channel is central to optogenetics and when expressed in the cellular membrane of a neuron it allows the neuron to be excited or inhibited, depending on the type of ion that flows through the channel, in response to light stimulation. The light stimulation is effective on the timescale of a neurons’ action potential, which is a few milliseconds. Two major systems of membrane protein expression are used to express the channelrhodopsin in the target cell, namely Cre recombinase expressing neurons in combination with targeted adeno-associated virus vector injections and the Cre-Lox recombination system (Madisen et al., 2012). In the latter two homozogous mice are crossed, one expressing Cre recombinase in target neurons and the other carrying the channelrhodopsin DNA flanked by loxP sites allowing the targeted expression of the channelrhodopsin membrane protein in the Cre expressing neurons.