Drumming with dopamine neurons
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Link to publication

Citation for published version (APA):

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CHAPTER 5

General discussion
5.0 Summary of results

5.0.1 Local interactions

In chapter 2 we analyzed possible interactions between the spontaneous activity of multiple simultaneously recorded dopamine neurons in the lateral VTA. Synchrony within the VTA was studied through the local interactions between VTA dopamine neurons, based on spike train statistics. These interactions were termed ‘functional connectivity’ and quantified with the Paired Phase Consistency (PPC) (Vinck et al., 2010). Under baseline conditions significant interactions were found with around 20% phase modulation compared to non-interacting neurons. Changes in functional connectivity (spike timing), were studied under the modulation of neuronal excitability (firing rate). The firing rate of the VTA dopamine neurons was modulated with high potassium and glutamate (300 µM). Both substances had a similar effect on the firing rate, but differential effects on the functional connectivity. High potassium reduced the functional connectivity, while it remained constant during glutamate administration.

This indicates that the functional connectivity is an emergent property of the population activity and not reducible to the spontaneous firing patterns of the individual VTA dopamine neurons. The timing structure, based on synchrony, thus represents a unique aspect of the VTA spike output received by its target brain areas.

5.0.2 Pharmacological network effects

Network interactions between VTA dopamine neurons were found in chapter 2. The relation between these emergent properties of the spontaneous population activity and the effects of pharmacology are largely unknown. Understanding network level effects of pharmacological substances could lead to a better understanding of drug (side-) effects.

In chapter 3 we studied pharmacological effects on dopamine neuron population activity and its network structure. A dopamine neuron releases dopamine into the extracellular space, depending on its activity. Through diffusion this activates the D2-receptor on the neuron itself and on surrounding dopamine neurons. We hypothesized that such volume transmission of dopamine (Cragg et al., 2001; Zoli et al., 1998) could affect the interactions within the local VTA network, as described in the introduction.

To this end, we defined the dopamine sensitivity of an individual VTA dopamine neuron as its EC50 for quinpirole, a dopamine D2-receptor agonist. The EC50 quantified the relation between D2-receptor activation and inhibition of neuronal firing. Granger causality was used to assess the directionality and strength of the interactions between pairs of VTA dopamine neurons. Our Granger causality analysis showed that VTA dopamine neurons play various roles in the local VTA network, from leading to following. These roles were related to their individual dopamine sensitivity (their sensitivity to the extracellular dopamine signal). Less dopamine sensitive neurons took a more leading role than sensitive neurons. This can be explained by the leaders contributing to the extracellular dopamine signal, without being inhibited by it. The dopamine sensitive neurons on the other hand are inhibited by the extracellular dopamine signal and thus follow the population activity. When we blocked the influence of the extracellular dopamine signal with the selective dopamine antagonist sulpiride the follower-leader structure in the network was abolished. Studying such pharma-
cological network effects increase our understanding of drugs targeting the dopamine system and the D₂-receptor.

5.0.3 Input resonance structures

In chapters 2 and 3 we found network interactions between the spontaneous rhythmic activity of lateral VTA dopamine neurons. *In vivo*, the lateral VTA receives input from multiple brain areas involved in the mesolimbic system, communicating with the basal forebrain (nucleus accumbens), striatum and the amygdala. In chapter 4 we wondered how the lateral VTA responds to these inputs. What are the input-output characteristics of the local VTA network? To this end, we used genetically modified mice which expressed an excitatory optogenetic ion-channel (Madisen et al., 2012) specifically in dopamine neurons (Smidt et al., 2012). To study the resonance properties of the VTA and thereby its input-output characteristics we used wide field laser pulse stimulation in combination with our MEA recordings.

The strength of synchronization to regular stimulation (2 Hz) depended on the intrinsic oscillation frequency of the dopamine neuron. Neurons having oscillation frequencies with a harmonic relation to 2 Hz exhibited stronger synchrony. The resonance frequency was defined as the laser frequency where the synchrony between laser and neuron was the strongest (highest PPC). The VTA dopamine neurons were seen to resonate most strongly at their intrinsic oscillation frequency. Similar scaling was seen with Poisson stimulation, but in this case sub-harmonics of the oscillation frequency were emphasized. These results describe the VTA network as a filter bank, with the filtering tuned at the oscillation frequencies of its constituent dopamine neurons. In addition, its a non-linear filter bank as the VTA’s response to regular (narrow-band) versus Poisson (broad-band) stimulation was different.

Poisson stimulation allows the whole VTA network to be stimulated effectively as a whole, as it is broad band input. The simultaneous drive by Poisson pulses leads to common resonance and neuron-neuron interactions within the lateral VTA. This emergent network state had self-organizing properties, as the VTA generated a frequency selective response under noise-like conditions. This selective resonance to noise input can be a mechanism by which the VTA acts as a pacemaker and generates an intrinsic rhythm, which it sends to downstream areas.

5.1 Comparison to previous work

5.1.1 Previous slice work

Most slice electrophysiology within the VTA has been performed using patch clamp (Khaliq and Bean, 2010) or extracellular (Werkman et al., 2001) recordings. Such studies record from one or two VTA dopamine neurons simultaneously and their conclusions are based on averaging the results of individual neuronal recordings. This allows the researchers to make statements about the electrophysiological properties of the VTA dopamine neurons, but obscures phenomena that originate from the interaction of multiple of these neurons. Our work records from a multiple regular firing conventional dopamine neurons in the lateral VTA simultaneously and aimed at discovering whether the individual neurons interact and synchronize their pacemaker activity. The VTA dopamine neurons were shown to interact by
modifying each others spike timing. The strength of the interaction correlated with changes in firing regularity (chapter 2). Building on this we found network level effects of dopamine pharmacology (chapter 3) and a non-linear input filter bank description of the VTA (chapter 4).

5.1.2 In vivo findings

Some concerns can be raised whenever using ex vivo brain slices to study network synchrony, because many connections are absent following the preparation procedures. On the other hand the brain slice allows studying the connectivity within a brain region in isolation. In vivo work has shown that the VTA is involved in complex interactions with the PFC and hippocampus (Fujisawa and Buzsáki, 2011), which are naturally absent in our experiments. However, Fujisawa and Buzsáki (2011) hypothesized that the VTA could be the pacemaker for a 4 Hz rhythm entraining the PFC and VTA. We studied this pacemaker role ex vivo, by recording from the VTA in isolation. Fujisawa and Buzsáki (2011) recorded higher firing rates (higher oscillation frequency) of the VTA dopamine neurons in vivo compared to our experiments. This difference could be due to several factors. The VTA dopamine neurons are expected to receive more inputs in vivo, which could lead to more excited states. Additionally, Fujisawa and Buzsáki (2011) recorded from the ventromedial part of the VTA dopamine neurons, containing non-conventional dopamine neurons that project to the PFC and have faster firing rates compared to the lateral VTA (Lammel et al., 2008).

5.1.3 Resonance network structures

Experimental work on entrainment and resonance analogues to Chapter 4 is scarce and our work is mostly inspired by modeling work, such as Hata et al. (2010). The functional importance of noise-induced-synchrony has been realized recently (Ermentrout et al., 2008; Galán et al., 2006). Their experimental work on olfactory bulb neurons found synchrony between neurons to common noise input. The synchrony was strongest at the average spontaneous firing frequency of the olfactory bulb neurons (~20 Hz, (Galán et al., 2006)). Their work builds on studies of (sub-threshold) resonance, such as described by Pike et al. (2000) and extends it by examining resonance and synchrony to common noisy inputs (Galán et al., 2006). Our work studies network interactions during noise-induced-synchrony in more detail and with larger neuron populations. Our findings of increased partial coherence during noise stimulation of the VTA indicates an emergent network state which is not solely based on the common noise input.

5.2 Mechanisms

5.2.1 The VTA’s functional connectivity

What underlies the functional connectivity between VTA dopamine neurons we measured in our studies? As described in the introduction (section 1.0.3) the VTA is known to contain various types of local synaptic connectivity between its dopamine, GABAergic and glutamatergic neurons. Chapter 3 added a mechanism by which VTA dopamine neuron can interact.
Dopamine volume transmission was found to organize the hierarchy of interactions between VTA dopamine neurons. We were not able to record GABAergic neuron activity in the VTA with our MEA technique, presumably due to their smaller size and electrical currents. Still, in chapter 2 experiments were performed with the GABA<sub>A</sub> receptor antagonist bicuculline with no effect on firing rate or functional connectivity. We could not examine the role of the GABAergic neurons in detail, but to the best of our knowledge they did not play a large role in the network interactions in our brain slice preparation.

5.2.2 Firing regularity and functional connectivity

In chapter 2 we used two well understood manipulations of neuronal activity: increasing extracellular potassium and bath-application of glutamate. Their effect on mean firing rate and mean oscillation frequency were similar, but they had a differential effect on firing irregularity (LV) as well as on functional connectivity. The increase in firing rate induced by high potassium correlated with a lower LV (increased regularity), while such correlation was absent in the case of glutamate application. What could be the mechanism underlying this finding? Depolarization of the membrane potential by high potassium can strengthen the role of the ‘persistent’ sodium current in the generation of spikes (Khaliq and Bean, 2010) and this can explain the reduction in firing irregularity (Drion et al., 2011). Glutamate depolarizes the neuron through the activation of post-synaptic AMPA and NMDA receptors (Wang and French, 1993). The strongly increased synaptic activity enhances firing irregularity (Drion et al., 2011) and could therefore explain the difference between the effect of glutamate- and potassium-induced depolarization. The weakening of neuronal interactions could be due to a change in the resonance properties of the neurons (Hunter et al., 1998; Coombes and Bressloff, 1999). The strengthening of the intrinsic rhythm by larger sodium currents could make the dopamine neurons less sensitive to synchronizing inputs. Indeed, this is a general property of oscillators. As an oscillator becomes more regular, the range of resonance frequencies gets narrower ((Pikovsky et al., 2002)). Thus, a perfect oscillator will by approximation only respond to inputs that respect its own rhythm.

5.2.3 Dopamine volume transmission

Volume transmission of neurotransmitters through the extracellular medium is a general mechanism of neuronal communication (Zoli and Agnati, 1996; Zoli et al., 1998). Experiments have shown volume transmission of dopamine within the SN (Vandecasteele et al., 2008). Modeling studies of dopamine volume transmission in the VTA and SN, suggest a significant role in neuron-neuron communication (Cragg et al., 2001). These models showed that the extracellular dopamine dynamics and diffusion are capable of influencing the activity of dopamine neurons in close proximity. The extracellular dopamine levels following dopamine neuron activation are in the concentration range of D<sub>2</sub>-receptor activation (Ford et al., 2009). Chapter 3 provides evidence for a role of dopamine volume transmission in organizing network interactions between dopamine neurons in the VTA. VTA dopamine neurons release dopamine into the extracellular space during neuronal activity. Additionally, they have extra-synaptic dopamine receptors to pickup this extracellular dopamine signal, which can lead to auto- and cross-inhibition of itself and nearby dopamine neurons. We found that the dopamine sensitivity, a measure for the effect of D<sub>2</sub>-receptor activation on the firing rate of
the neuron, correlated with its role (follower or leader) in the local network. We can explain this correlation with volume transmission; the more dopamine sensitive neurons are more affected by the extracellular dopamine signal and follow its dynamics, which are generated from the summed activity of nearby dopamine neurons.

5.2.4 Input resonance

In chapter 4 we found sub-harmonic resonance to Poisson pulses. Although we did not study the underlying mechanisms directly, the resonance is likely due to sub-threshold oscillations (Lampl and Yarom, 1997) described in various models of the VTA dopamine neurons (Kuznetsov et al., 2006; Medvedev et al., 2003). We hypothesize that the sub-harmonic resonance we find can be understood by the stochastic drive of an non-linear oscillator (Tiesinga, 2002; Hata et al., 2010), which has a preferred stimulus phase within the spike cycle (i.e. just before spike threshold). This preferred stimulus phase was clearly seen in the phase-response-curves of the VTA dopamine neurons. Poisson stimuli are less probable to fall within this preferred time window each spike cycle. It is more likely the Poisson stimuli will be effective every other cycle or every third (sub-harmonics). Thus a stochastic drive will push the neuron with sub-harmonics of its oscillation frequency on the basis of probability.

5.3 Functional implications

5.3.1 Network effects of pharmacology

The main goal of our study was to understand the local network interactions among dopamine neurons in the lateral VTA. In chapter 2 we described functional connectivity between dopamine neurons being modulated by increased extracellular potassium. The different modulation of functional connectivity by glutamate and potassium excitations gave a first impression of emergent network effects of simple manipulations on the neuronal level. Chapter 3 took the next step and combined traditional dopamine pharmacology with network activity analysis. The roles the dopamine neurons play in their surrounding network, from follower to leader, were organized based on the sensitivity of their firing rate to D2-receptor activation. This means that most antipsychotics (which are dopamine D2-receptor antagonists) likely have emergent network effects that can not be reduced to their effect on individual dopamine neurons. These network effects are not well studied and could lead to a better understanding of pharmacological effects on the VTA in relation to schizophrenia and other disorders.

5.3.2 The VTA a 4 Hz pacemaker?

Fujisawa and Buzsáki (2011) proposed the VTA as a candidate for the pacemaker of a 4 Hz rhythm which entrains the PFC with the VTA. Can the VTA be the pacemaker of this mesocortical oscillation? In chapter 2 we found that individual VTA dopamine neurons have in intrinsic oscillation frequency between 1-4 Hz. Pairs of dopamine neurons were seen to interact and this functional connectivity was strongest at their oscillation frequencies. Chapter 4 analyzed this in detail and described the entrainment properties of a VTA dopamine neuron peaking at its oscillation frequency for regular pulsed inputs. A description of the VTA as
a non-linear filter bank was also arrived at in chapter 4. The VTA dopamine neurons were found to resonate and synchronize at sub-harmonics of their oscillation frequencies in response to common Poisson pulse stimulation. The resonance characteristics to noisy input could give the VTA its pacemaker function. If driven with noisy input the VTA enters a network state in which the VTA dopamine neurons output specific frequencies. In our experiments these output frequencies lay between 0.25-2 Hz, which were subharmonics of the oscillation frequencies we measured (1-4 Hz). Fujisawa and Buzsáki (2011) reported oscillation frequencies up to 10 Hz for PFC projecting dopamine neurons corroborating Lammel et al. (2008). In this case sub-harmonics in the VTA output could reach the reported 4 Hz pacemaker rhythm. Interestingly, our findings suggest that the mesolimbic system could be driven by a 1-2 Hz oscillator, a sub-harmonic of the slower firing conventional mesolimbic projecting dopamine neurons.

5.3.3 Time coding by the VTA filterbank

The VTA has long been implicated in stimulus-reward coupling, with reward prediction based on an internal time code. VTA dopamine neurons phasic activity is proposed to act as a reward prediction signal, which is then modulated by GABAergic VTA neurons that receive external input Cohen et al. (2012). The underlying timing structure of the reward prediction is crucial in this concept. However, the neural substrate of such a time code in the range of seconds is as yet unknown Schultz (1997); Lak et al. (2016). The entrainment of VTA dopamine neurons to common rhythmic pulse input could serve a function in establishing such a time code. The VTA dopamine neurons determine the time code based on their oscillation frequency, which defines their entrainment characteristics. Our results suggest that the lateral VTA acts like a filter bank, whose neuron sub-populations resonate to different frequencies in the external input. The neurons in these sub-populations are organized by having a similar or harmonically related intrinsic oscillation frequency. The output of these oscillating sub-populations encode the timing information in the input and could be used as a time code by down-stream target areas of the lateral VTA. Input with the right timing structure will be passed to VTA projection areas, while unaligned input will not entrain the VTA and will not be passed downstream.

5.3.4 Hierarchy within the VTA

Granger analysis allowed us to peer into the structure of network interactions within the VTA. We found asymmetric interactions between dopamine neurons within the VTA. Some neurons behaved as 'leaders' and exerted a strong Granger-causal influence on the activity of other cells in the network, while receiving a smaller Granger-causal influence from these other cells in turn. For other cells the opposite held true ('follower cells'). We can speculate that the leading dopamine neurons receive input from upstream areas, which is different from the followers. For instance, if glutamatergic projections to the VTA target the leaders. These leaders will integrate this input and control the timing of nearby followers creating a multi-layered network of neurons within the VTA. One layer receiving input and a second layer following and incorporating the spike timing of the other layer. Such multi-layered networks have been studied intensively. In general, the more layers a network has, the more complex and flexible the computations within the network can be (Meunier et al., 2010).
5.3.5 Emergent network phenomena

Generating hierarchical levels of abstraction has been proposed to be the brains’ main function (Hofstadter, 2008). An abstraction layer is a way of reducing complexity by hiding details between two levels of (symbolic) processing, a concept which has fueled the digital revolution (Agarwal and Lang, 2005). For instance, the abstraction of the digital 0 and 1 hides the details of the analogue components below, such as transistors and capacitors. A neurons’ soma is a good example of an abstraction layer in the brain. The soma combines input from the dendrites and conditionally fires an action potential along the axon. The action potential output hides almost all details of the dendritic summation of synaptic input. It has abstracted away the complexity of temporal and spatial occurring synaptic events from the downstream neurons which receive only a synaptic event (IPSP/EPSP) as a consequence of the presynaptic action potential.

The functional connectivity and resulting network states we studied in this thesis are emergent phenomena of the dopamine neuron population activity, as they can not be understood by studying the activity patterns of individual dopamine neurons. Such emergent phenomena could be a mechanism with which the brain abstracts away the underlying details of neuronal activity. Similar to the digital bit and the action potential, these network states are conceptually separate from their underlying detailed mechanics.

5.4 Outlook and further directions

5.4.1 The mystery of the GABAergic neuron

The VTA is known to constitute of around 30% GABAergic neurons, which make local synapses with dopamine neurons (Nair-Roberts et al., 2008; Omelchenko and Sesack, 2009). With our MEA recordings we were not able to pick up these neurons, likely due to their smaller soma size and resulting electrical presence. We could therefore not assess the role they play in the lateral VTA network. Our experiments with the GABA_A antagonist bicuculline did not gain us insights thereof. In the future the MEA could be combined with sharp electrode recordings of these GABAergic neurons to assess their relation to the dopamine neuron population activity. A similar Granger causality analysis as in chapter 3 could be used to assess whether the GABAergic neurons are leading or following.

5.4.2 In vivo research and further directions

In vivo the brain is intact and our results could be understood in the context of the fully connected VTA. The VTA could be interrogated by stimulating the axons projecting into the lateral VTA or by optogenetically stimulating upstream brain areas. The entrainment and resonance properties of the VTA we described could then be studied as a non-linear filter bank or even a pacemaker node within a network of brain areas containing the PFC, NAc and hippocampus and others. In addition, we could study the hierarchy in the VTA dopamine neuron network. Do leaders receive different input from followers? Can we view the leaders and followers as two layers in a micro-circuit?
HET VENTRALE TEGMENTUM (VT) IS EEN DOPAMINEKERN IN HET MIDDENBREIN, WAT ÉÉN VAN DE EVOLUTIONAIR OUDERE DELEN IS VAN HET BREIN. DOPAMINE IS EEN BELANGRIJKE NEUROTRANSMITTER IN HET BREIN EN SPEELT O.A. EEN ROLE IN VERSLAVING, DE ZIEKTE VAN PARKINSON EN SCHIZOFRENIE. DEZE DOPAMINEKERN IS IN VERBAND GEBRACHT MET DIVERSE HOGERE COGNITIEVE TAKEN ZOALS GEHEUGEN EN LEREN (KIM ET AL., 2012; FUJISAWA AND BUZŚAKI, 2011) NAAST ZIJN ROLE IN HET BELONINGSSYSTEEM (SCHULTZ, 1997).

Het VT bestaat voornamelijk uit dopamine neuronen, naast GABA-neuronen. Deze dopamine neuronen zijn spontaan actief en generen actiepotentialen in rusttoestand. Deze activiteit is ritmisch en laag frequent (1-5 Hz). Voor leren en geheugen blijkt niet alleen de activiteit van de dopamine neuronen belangrijk, maar ook de onderlinge samenhang van deze activiteit (correlatie). In dit proefschrift beschrijven we een onderzoek naar het lokale VT netwerk in isolatie, om beter te begrijpen hoe het VT intern werkt en zijn diverse rollen vervult. Het gebruik van een hersenplakjespreparaat in combinatie met een 'Multi-Electrode-Array' (MEA, (Taketani and Baudry, 2010)) maken metingen binnen het VT mogelijk zonder invloeden van buitenaf, zoals activiteit van andere hersengebieden.

In hoofdstuk 2 beschreven we de samenhang binnen de VT dopamine neuron populatiactiviteit tijdens rusttoestand. Deze samenhang noemden we 'functionele connectiviteit' en kwantificeerden we met de 'Paired Phase Consistency' (Vinck et al., 2010). Er bleek significante functionele connectiviteit te zijn tussen dopamine neuronen. Op basis hiervan onderzochten we of het algemene activiteitsniveau van het VT invloed had op de samenhang van de activiteit, door middel van het verhogen van de extracellulaire kalium concentratie en het toedienen van glutamaat. Beiden verhoogden de activiteit op een vergelijkbare manier, maar hadden verschillend effect op de functionele connectiviteit. Namelijk, de sterkte van de
connectiviteit nam af tijdens de verhoging van de kalium concentratie, maar bleef gelijk onder toediening van glutamaat. Dit betekent dat de functionele connectiviteit een eigenschap is van het lokale VT netwerk, die grotendeels los staat van de activiteit van het VT. Kennis van deze functionele connectiviteit vergroot dus het begrip van het VT en van de effecten van farmacologie gericht op het VT.

In hoofdstuk 3 beschreven we de interne samenhang van de activiteit in het VT in relatie tot farmacologische manipulatie, namelijk activatie van de dopamine D2-receptor. Dopamine neuronen heten zo omdat ze dopamine produceren en uitscheiden. Dit doen ze wanneer ze geactiveerd worden. De dopamine komt terecht in de ruimte tussen de neuronen (extracellulaire ruimte) en beïnvloedt andere nabijgelegen neuronen door dopamine receptor activatie. We stelden vast dat de dopamine neuronen in het laterale VT ieder hun eigen gevoeligheid hebben voor dopamine. Deze gevoeligheid bepaalde vervolgens de rol van het dopamine neuron in de omliggende dopamine neuronpopulatie. De gevoelige neuronen werden meer beïnvloed door de ongevoelige neuronen met dopamine in de extracellulaire ruimte als communicatiesignaal (volumetransmissie, (Zoli et al., 1998)). De gevoelige neuronen waren dan ook te typen als volgers en de ongevoelige neuronen als leiders, wat we kwantificeerden met de Granger causality analyse (Granger, 1969). Het bleek dat D2-receptor activatie niet alleen effecten heeft op de individuele dopamine neuronen (vuurfrequentie), maar dat ook de onderlinge samenhang werd beïnvloed. Deze effecten van receptor activatie op het netwerk niveau kunnen mogelijk verklaren waarom medicijnen gericht op het dopaminesysteem, zoals antipsychotica, zo moeilijk te ontwikkelen zijn. Het bestuderen van zulke meta-effecten van farmacologische manipulatie kan leiden naar beter inzicht in de (bij)effecten van medicijnen gericht op het dopamine systeem.

In hoofdstuk 4 namen we een actievere rol in de experimenten. In plaats van alleen passief de spontane activiteit van het VT te meten, stimuleerden we nu de dopamine neuronen met een hoge temporele precisie via optogenetica. Optogenetica is het genetisch modificeren van neuronen waardoor zij een lichtgevoelig ionkanaal in hun celmembraan hebben (Deisseroth, 2010). Hierdoor is het neuron met een laser te activeren, waardoor er een stroom gaat lopen over het celmembraan, wat een actiepotential kan genereren. Dit stelde ons in staat meer gedetailleerde en krachtigere onderzoeksvragen na te jagen. Ons eerste doel was het gedetailleerder bestuderen van de functionele connectiviteit. Hierbij was de oscillatiefrequentie van het dopamine neuron belangrijk. Dit is de natuurlijke frequentie van de spontane ritmische activiteit van een dopamine neuron (1-5 Hz). Door pulsstimulaties van verschillende frequenties aan te bieden konden we bepalen op welke frequentie het neuron ging resoneren en daardoor ging synchroniseren met de laserpulsen. Deze optimale frequentie bleek dezelfde als de oscillatie frequentie van het neuron. Zoals bij een slinger van een klok vond resonantie dus plaats op de eigen frequentie van het neuron. De collectie van dopamine neuronen in het VT met hun verschillende oscillatiefrequenties kan dus gezien worden als een collectie oscillatoren met verschillende overlappende frequentiekarakteristieken. Dit is relevant voor het coderen van timing informatie in de input naar het VT en 'stimulus-reward' voorspelling (Schultz, 1997).

Een nieuw principe in dergelijk resonantie- en synchronie-onderzoek is 'noise-induced-synchrony' (Ermentrout et al., 2008), waarbij neuronen onderling synchroniseren doordat zij door dezelfde ruis (random) stimulus worden aangedreven. Deze vorm van synchronisatie is van belang omdat het brein per definitie 'noisy' is en verwerking van ruis mogelijk gezien kan worden als een eigenschap en niet als storing. Door onze laserpulsen op random (Poisson)
momenten aan te bieden met een gemiddeld aantal (5) per seconde, konden wij de synchro-
nisatie binnen het VT bestuderen onder 'noisy' omstandigheden. De VT dopamine neuro-
men resoneerden met de half-frequentie (sub-harmonisch) van hun oscillatiefrequentie tijdens
Poisson stimulatie, lager dan bij regelmatige stimulatie. Dit liet zien dat het VT een collectie
van niet-lineaire filters is, waarbij regelmatige stimulatie anders wordt gefilterd dan Poisson
stimulatie. Daarnaast ontstond er tijdens Poisson stimulatie een toestand van het netwerk
met emergente eigenschappen. Namelijk, er vond 'noise-induced-synchrony' plaats in het
VT met emergente netwerk interacties, wat een unieke meting is. Een dergelijke meting is
maar enkele keren eerder gedaan (Ermentrout et al., 2008). De netwerktoestand ontstaat door
de frequentie selectieve resonantie van de dopamine neuronen met de 'noisy' input. Hier-
door creëren de VT dopamine neuronen structuur op basis van 'noisy' input. De frequentie
selectieve output van VT dopamine neuronen onder 'noisy' stimulatie kan een mechanisme
zijn waardoor het VT de laag frequente 'pacemaker' rol kan vervullen naar de hypothese van
Fujisawa and Buzsáki (2011). Hierbij zal het VT dienen als een gangmaker van een ritme
dat belangrijk is voor leren en geheugen, wat zowel de hippocampus en de prefrontale cortex
synchroniseert.

Kortom, in dit proefschrift zijn we er in geslaagd de lokale organisatie van neuronale in-
teracties in het VT te bestuderen. Door een combinatie van technieken hebben we fenomenen
als functionele interactie, resonantie en netwerktoestanden kunnen meten en manipuleren.
Dergelijke emergente eigenschappen van de activiteit in het VT gaan een grote rol spelen
in verder onderzoek naar de (bij)effecten van medicijnen gericht op het dopamine systeem.
Verder kunnen zij het begrip vergroten van de cognitieve functies van het VT, met betrekking
tot leren, geheugen en beloning.