Drumming with dopamine neurons
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Self-organizing properties and non-linear filtering in the mouse Ventral Tegmental Area neuronal network in-vitro

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van der Velden, L., Vinck, M. & Wadman, W.J. The VTA network, a non-linear filter bank with time coding and self-organizing properties
Abstract

The ventral tegmental area (VTA) is a dopaminergic nucleus in the midbrain with the propensity to exhibit spontaneous intrinsic rhythmic activity in the 1-5 Hz frequency range and can be considered as a network of weakly coupled oscillators. Here, we combine in-vitro simultaneous action potential recording from a 60 channel multi-electro-array with optogenetic stimulation of specific dopaminergic VTA neurons to investigate how VTA neurons filter wide-band stochastic as well as deterministic input, and how they encode it into their spontaneous oscillatory output. The dopaminergic neuron population was activated either by fixed frequency laser pulse stimuli in the same frequency range as the intrinsic oscillations or by stochastic Poisson distributed stimulation of similar mean frequency. These stimuli affected spike timing much more than firing rate and strong synchrony was seen between the rhythmic laser input and the spike timing of the neurons. For rhythmic pulsed inputs, neurons showed resonant behavior with the strongest phase locking at their intrinsic oscillation frequency. For stochastic Poisson pulse stimulation, which provided a more effective stimulation of the entire population, we observed resonance at lower frequencies than the intrinsic oscillation frequency. Poisson stimulation induced an emergent network state, which rapidly switched between stimulation on and off. The non-linear filter characteristics of its dopamine neurons allow the VTA to encode timing information in its input, relevant for stimulus-reward prediction. In addition the noise-induced-synchrony endows the VTA with self-organizing properties with which it could generate pacemaker output on the basis of noise-like input.
4.0 Introduction

The ventral tegmental area (VTA) is a dopamine nucleus in the midbrain, alongside the substantia nigra and the red nucleus. VTA dopamine neurons exhibit spontaneous low-frequency rhythmic spike activity (Werkman et al., 2001; Bayer et al., 2007). The rhythm is intrinsic and is even retained in isolated VTA dopamine neurons (Koyama et al., 2005). Experimental research and modeling studies point to at least two distinct mechanisms underlying this oscillatory activity (Khaliq and Bean, 2010; Drion et al., 2011): either sub-threshold calcium oscillations or the persistent sodium current. VTA dopamine neurons have direct synaptic connections among each other; they connect through a local interneuron network (Bayer and Pickel, 1990; Omelchenko and Sesack, 2009) and they likely interact through dopamine volume transmission (Cragg et al., 2001). The VTA network is therefore best considered as a network of weakly coupled oscillators.

Oscillatory rhythms in the brain create the context for encoding information, specifically in their detailed timing (Buzsáki, 2009). The VTA is implicated in a 4 Hz local field oscillation which synchronizes it with the prefrontal cortex and the hippocampus during memory processing (Fujisawa and Buzsáki, 2011; Battaglia and McNaughton, 2011). An additional hypothesis states that VTA pacemaker activity entrains the prefrontal cortex and the hippocampus (Fujisawa and Buzsáki, 2011). The timing information in its output likely plays an important role in stimulus-reward processing (Schultz, 1997) especially where activities need to be related at relatively long time scales.

Studying the population dynamics of the VTA dopamine neurons requires simultaneous recording of the spiking activity of as many neurons as possible, which can be accomplished in an acute brain slice positioned on a multi-electrode-array (MEA). The slice is void of external input, which restricts the preparation, but also prevents complications from unquantifiable external input and thus simplifies the analysis. Specific manipulation of neurons at a very fast time scale can be accomplished with optogenetics. The Ptx3 Cre driver mouse (Smidt et al., 2012) was used to express light sensitive excitatory channelrhodopsin in dopamine neurons, by crossing it with the floxed ChR2-YFP mouse (Madisen et al., 2012). Pulsed laser driven activation then allows detailed manipulation of spike timing at the level of individual neurons as well as for the entire population (Deisseroth, 2010; Fenno et al., 2011) during long-term recordings without noticeable photo-toxicity (Cardin, 2012).

This study focuses on the lateral VTA, which mainly contains mesolimbic projecting dopamine neurons with a classic dopamine neuron phenotype (Lammel et al., 2008). The auto-oscillatory VTA dopamine neuron response to rhythmic input was investigated and we probed their resonance characteristics at the population level with regular and Poisson pulsed (noisy) input. Driving populations of oscillators with such stimulus regimes has theoretically been worked out and is able to reveal interesting dynamics (Hata et al., 2010). Experimental and modeling research have shown the ability of populations of oscillators to encode common input into their oscillatory output, even when the input is noisy (Ermentrout et al., 2008). We show here that the resonance characteristics of the VTA dopamine neuron population revealed with temporally patterned stimulation demonstrate emergent properties at the network level, which could well support the higher level functions proposed for the VTA.
4.1 Methods

4.1.1 Experimental animals

Adult male and female mice between 6 and 11 weeks old were used in the experiments. They were housed in a 12/12 light dark cycle and received water and food ad libitum. All procedures and methods were approved by the ethical committee for animal experimentation of the University of Amsterdam.

4.1.2 Optogenetic expression

Expression of the ChR2 optogenetic channel was realized with the Cre-Lox system. Two strains of mice: the Ptx3-Cre driver (Smidt et al., 2012) and the Lox-ChR2-YFP mouse (Madisen et al., 2012) were cross-bred. Both had a black-6 genetic background and were homozygous for their respective allele of interest. First generation offspring, heterozygous for both alleles (male and female), were used in the experiments. Selective expression of Ptx3 in dopamine neurons was extensively reported in the VTA, the Substantia Nigra and the Red Nucleus (Smidt et al., 2000, 2012).

4.1.3 Slice preparation and electrophysiology

Mice were decapitated, the midbrain was dissected and kept in artificial cerebral spinal fluid (ACSF) at 4 °C, containing (in mM) NaCl 120, KCl 3.5, CaCl$_2$ 2.5, NaH$_2$PO$_4$ 1.25, MgSO$_4$ 1.3, NaHCO$_3$ 25, D-glucose 10 which was bubbled with carbogen (95 % O$_2$; 5 % CO$_2$) to set pH at 7.4. Coronal slices were cut 300 μm thick from caudal to rostral using a vibratome (Leica VT1000S, Wetzlar Germany). The fading of the substantia nigra during progressive slicing was a marker for the caudal-medial part of the VTA. One or two slices containing the caudal and medial part of the VTA were used for the experiments. Slices were incubated for 30 minutes at 32 °C directly after slicing and were kept at room temperature until the start of the experiment. During recording (MEA-1600, Multichannel Systems, Reutlingen, Germany) the slice was kept at 32 °C and continuously perfused with ACSF bubbled with carbogen. The VTA was identified in the mid-brain slice and positioned on top of the 3D MEA (Qwane Biosciences, Lausanne, Switzerland) containing 60 electrodes (8*8 layout) of 30 μm diameter and 100 μm spacing in order to record the spontaneous activity of multiple single-units (Olivier et al., 2002). All chemicals were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands).

4.1.4 Data acquisition

The extracellular MEA recordings showed identifiable extracellular spikes of 30 to 130 μV amplitude superimposed on a background noise of about 15 μV. The raw signal was high pass filtered at 225 Hz using a second order Butterworth filter and sampled at 20 kHz. Positive voltage peaks were detected, with a relatively low threshold in order not to miss spikes. Negative polarity spikes were rarely recorded and less suitable for analysis due to the negative polarity photoelectric artifact of the laser pulse stimulation on the MEA. Patches of signal containing the peak waveforms (±3 ms around the peak) were collected. K-means
clustering was used to define the largest two principle components and the peak amplitudes of the waveforms. The auto-correlation and inter-spike-interval distribution of the peaks in the clusters were examined to identify clusters consisting of neuronal spikes. For electrodes that contained more than one neuron the most reliably recorded neuron was selected, based on peak amplitude.

### 4.1.5 Laser stimulation protocols

A 532 nm laser (GL532T3, Shanghai Laser & Optics Century Co., China, max. output: 320 mW) coupled to a 100 µm glass fibre through an optic coupler (OZ optics, HPUC-23AF, Ontario, Canada). Laser pulse stimulation was aimed from above, penetrated the slice until it reached the MEA at the bottom. A Raspberry-Pi micro-controller (Raspberry-Pi foundation, UK) was programmed in Python to digitally control the timing of the laser stimuli (duration always 10 ms) with a time resolution of around 20 µs. Two main protocols were used. The first one consisted of six 240 s periods of stimulation on or off using either fixed 2 Hz stimuli or Poisson distributed stimuli (exp(-λt)) with an expectation value per unit time λ of 5 events/s. The second protocol consisted of 30 s periods of laser stimuli with a frequency that could take values between 0.5 and 5.5 Hz in 0.5 Hz steps (random permutation).

### 4.1.6 Spike train analysis

The Peri-Stimulus-Time-Histogram (PSTH) gives the spike count in 5 ms bins around the laser stimulus (given at t = 0 accumulated over all stimuli. The spike probability in a bin was calculated as the fraction of laser stimuli that successfully induced a spike in that bin.

VTA dopamine neuron rhythmic firing is reflected in peaks in their auto-correlation function. The oscillation frequency quantifies the preferred intrinsic rhythm of the neuron and was computed from the mean interval between the side-lobes in the auto-correlation, including the interval between the zero-lag and the first side lobe.

The Phase-Response-Curve (PRC) measures the perturbation of a neurons’ spike cycle, due to the laser pulses. Taking the oscillation frequency as a reference, the phase of the spike cycle was normalized between zero and one. A laser pulse could either delay the time to the next spike (negative phase shift) or advance it (positive phase shift).

The strength of the synchrony between a neurons’ spike train and the laser pulses was quantified with the Paired-Phase-Consistency (PPC) as previously defined (Vinck et al., 2010). The PPC calculates the similarity of the relative phases of the point processes with respect to a chosen reference frequency and estimates the square of the classic Phase-Lock-Value (Lachaux et al., 1999). The PPC is an unbiased metric of phase-synchronization that scales with the square rather than the square root of the coherence and phase locking value (Vinck et al., 2010). To compute the PPC, spike trains were binned at 1 ms bins and a (Hanning) windowed Fourier Transform was computed on a series of time segments of the spike train. The length of the time segments was set to contain a fixed number of cycles of the reference frequency of interest (e.g. 5 cycles). The relative phase is defined as the complex argument of the classic spectral coherence (Lachaux et al., 1999; Vinck et al., 2010). From these relative
phases the PPC was computed:

\[
PPC = \frac{2}{N(N-1)} \sum_{j=1}^{N-1} \sum_{k=j+1}^{N} \cos(\theta_j - \theta_k)
\] (4.1)

where there are \(N\) time segments, segment \(j\) has relative spike phase \(\theta_j\) and segment \(k\) has relative spike phase \(\theta_k\), computed in respect to a chosen reference frequency. For the interleaved 2 Hz and the Poisson protocol we computed the PPC at selected frequencies independent of the laser pulse frequency. In the stepped frequency protocol we computed the PPC only at the frequencies of the laser stimuli. The PPC was also used to assess the synchrony between spike trains simultaneously recorded from pairs of neurons.

To disentangle the synchrony between neurons driven by common laser input and the part due to direct interactions between the neurons, we computed the partial coherence (Rosenberg et al., 1989, 1998; Brett et al., 2009). The spectral estimation for the coherence computation was performed using welch’s algorithm with overlapping 5 s windows (Welch, 1967; Brett et al., 2009).

Rolling correlations were computed between the activity of individual dopamine neurons and the laser pulses with a window length of 5 s (McKinney, 2010). When quantifying the transition between laser on and off states we computed the correlation during stimulation off from virtual laser stimuli with the same timing as the real ones (\(\lambda = 5\), Fig.4.7A).

4.1.7 Statistics

Unless otherwise mentioned, all values reported in this study are given as mean and standard error of the mean (mean ±SE). In graphs the area indicated around a line indicates ±SE. Unless otherwise mentioned, direct comparison of two means was performed with Student’s t-test, after checking for normality. Comparison of multiple groups was performed with ANOVA and post-hoc testing. The variance of the PPC for individual neurons was estimated with a jackknife method for each frequency of interest. The variable \(n\) is used to indicate the number of observations, usually the number of slices, which equals the number of animals. \(P < 0.05\) was used to reject the null hypothesis.

4.1.8 Immunocytochemistry

Co-expression of the two genes in the VTA dopamine neurons was checked in the F1 offspring, heterozygous for both alleles, using fluorescent immuno-staining with GFP and Ptx3 (Smidt et al., 2000) anti-bodies, combined with confocal and wide field imaging. Free floating acute brain slices (100 \(\mu\)m), were fixed for 1 hour at room temperature with 4 % paraformaldehyde in phosphate buffered saline (PBS) pH 7.4. Subsequently washed with PBS and blocked for 4 hours with 10 % normal goat serum + 0.25 % Triton X-100 in PBS at room temperature. The slices were incubated overnight with the primary antibodies GFP (1:750; Abcam; ab13970) and Ptx3 (1:1000; (Smidt et al., 2000)) diluted in 3 % normal goat serum + 0.25 % Triton X-100 in PBS at 4 °C. The following day, the slices were washed with PBS and incubated for 4 hours with the secondary antibodies Goat-anti-Rabbit- Alexa Fluor 555 (1:1000; Molecular Probes; A-21428) and Goat-anti-Chicken- Alexa Fluor 488 (1:1000; Molecular
Probes; A-11039) diluted in 1% normal goat serum + 0.025% Triton in PBS at room temperature. Sections were washed with PBS and cover slipped with Vectashield including DAPI (Vector Laboratories, Burlingame, U.S.). The co-localization of the excitatory ChR2 optogenetic channel and Ptx3 was checked with immunological staining. Both ChR2-Yfp and Ptx3 were selectively expressed within dopamine nuclei in our midbrain preparation (SI Fig. 4.1A), the SNpc and the VTA. Confocal images showed Ptx3 expression within the soma (red) combined with ChR2-Yfp expression (green) on the membrane, which lead to yellow coloring (SI Fig. 4.1B). To characterize the co-expression, Z-stack images were acquired using a Zeiss LSM510 confocal laser scanning microscope fitted with a 63x objective. The orthogonal views enhanced the view of the co-localized expression (SI Fig. 4.1B).

Figure 4.1: Expression of the excitatory optogenetic channel. A, Representative wide-field micrograph showing the expression pattern of both YFP (green) and Ptx3 (red) throughout the midbrain. One lateral side of a coronal midbrain slice is shown (SNc and lateral VTA). Scale bar represents 50 µm. B, Representative orthogonally projected confocal micrograph showing a YFP+/Ptx3+ neuron located in the VTA. Co-localization of Ptx3 expression (red) and ChR2-YFP (green) is seen as a yellow coloring of the neuronal membrane. The orthogonal side views further illustrate the membrane expression of ChR2-YFP. Scale bar represents 10 µm.
4.2 Results

4.2.1 Spontaneous activity and modulation by laser stimuli

VTA dopamine neurons fired spontaneous rhythmic action potentials (spikes). Fig. 4.2C shows the distribution of the intrinsic oscillation frequencies of the recorded neurons. Their values varied between 1 and 5 Hz; the variation was related to the neurons within a slice and not different between experiments (ANOVA, $n = 7$, $p = 0.56$).

The raster plots in Fig. 4.2A,B show short spike trains (excerpts out of the six 240 s periods) simultaneously recorded from seven VTA dopamine neurons under regular 2 Hz (Fig. 4.2A) laser stimulation and Poisson distributed ($\lambda = 5$, Fig. 4.2B) laser stimulation. The data suggests that after switching the laser pulses on ($t > 0$) neurons more readily entrain to regular than to Poisson stimuli. The PSTH of an example neuron during 2 Hz regular stimulation (Fig. 4.2D), showed an increase in spike occurrence just after laser pulse onset ($t = 0$) immediately followed by a relative decrease. This observation was repeated for all recorded neurons ($n = 73$ from 7 experiments). From the peak of the PSTH of each neuron (black dot in Fig. 4.2D) we calculated the efficacy of the laser stimulation for inducing a spike: the spike probability of the laser pulse. This spike probability showed variation across neurons, but it was not different between experiments (ANOVA, $n = 7$, $p = 0.58$, Fig. 4.2E). We related the observed spike probability to the potential increase in firing rate that resulted from the stimuli (Fig. 4.2F) but found no relation (Fig. 4.2F, Spearman’s $\rho = 0.16$, $p = 0.17$). We conclude that laser stimulation affected spike timing rather than
Figure 4.2: Spontaneous activity and modulation by laser stimuli. A, Raster plot of simultaneously recorded spike activity of seven VTA dopamine neurons before and after laser onset (at \( t = 0 \), red line), with (2 Hz) laser stimuli (green arrows). B, Same as A, but now with Poisson distributed stimuli (5 events/s). C, Distribution of oscillation frequencies of all recorded VTA dopamine neurons (\( n = 73 \) from 7 experiments (=animals). D, Peri-Stimulus-Time-Histogram (PSTH) of an example neuron during 2 Hz regular stimulation. A peak is followed by a trough in the spike count during and after the laser stimulus (\( t = 0 \), stimulus duration of 10 ms). E, The peak value of the spike probability induced by the laser across neurons (\( n = 73 \)). Showing that the efficacy of the laser varies across neurons. F, the change in firing rate between the stimulation off and on conditions did not correlate with the peak spike probability induced by the optogenetic stimulation, indicating the absence of a firing rate effect.
4.2.2 Neuronal synchronization to the laser pulses

The spike timing modulation by both stimulation regimes (Fig. 4.3A,B) was quantified with the Phase-Response-Curve (PRC), which assesses the change in the timing of the next spike as function of the phase at which the stimulus was applied (normalized between 0 and 1, Fig. 4.3). The PRC for the 2 Hz stimulation mainly displayed a shortening of the spike cycle (positive phase shift, Fig. 4.3C). Poisson stimulation (Fig. 4.3B) induced roughly the same PRC (Fig. 4.3D). This data indicate that the laser stimuli lead to synchrony between laser pulse and spike.

This synchrony was quantified using the Paired-Phase-Consistency (PPC). One example neuron with an oscillation frequency close to 2 Hz synchronized strongly to the 2 Hz laser stimulation (high PPC at 1:1 mode, green line in Fig. 4.4A) including a higher harmonic at 4 Hz. Poisson stimulation lead in the same neuron to weaker, synchrony at a much lower frequency (one fourth of its oscillation frequency, blue line in Fig. 4.4A). A second neuron oscillating twice as fast (4 Hz), also synchronized strongly to the 2 Hz regular stimulation (1:2 mode, green line in Fig. 4.4B). The same neuron driven by Poisson pulses synchronized to half its oscillation frequency or even lower (2 Hz, blue line in Fig. 4.4B). In a third neuron an interesting alternative mode was found: it oscillated at 3 Hz and entrained to the 2 Hz laser (Fig. 4.4C). This polyrhythmic 2:3 mode was not present for Poisson stimulation where the neuron resonated most strongly at one fourth of its oscillation frequency (0.75 Hz, blue line Fig. 4.4C). The harmonic structure in these PPC spectra suggests a relation between neuronal resonance frequencies and the intrinsic oscillation frequency of the neurons.
4.2.3 Synchrony: neuronal resonance and filtering

The step frequency protocol that sampled stimulation frequencies between 0.5 and 5.5 Hz with 0.5 Hz resolution assessed how the peak resonance frequency relates to each neuron’s oscillation frequency. Each frequency was applied 6 times for 30 s and the frequency with the highest PPC value was scattered against the oscillation frequency (Fig. 4.5A). The neurons synchronized most strongly at their oscillation frequency (1:1 mode, black line in Fig. 4.5A). There was significant correlation between the peak resonance frequency and the oscillation frequency (Spearman’s \( \rho = 0.31, p = 0.008 \)). The analysis was repeated for Poisson distributed stimulation applied for about the same time period (\( \lambda = 5 \)). Neurons also resonated to Poisson stimulation, be it with lower frequencies (half their oscillation frequency and lower Fig. 4.5B). The PPC peak resonance frequency also correlated with the oscillation frequency during Poisson stimulation, but at a subharmonic (Spearman’s \( \rho = 0.57, p < 0.001 \), Fig. 4.5B, 1:2 line). We conclude that the preferred resonance frequency scales with the oscillation frequency of the VTA dopamine neuron but also that regular and Poisson stimulation regimes emphasized different resonance modes (1:1 for regular versus 1:2 and lower for Poisson stimulation).

These results are summarized in Fig. 4.5C, where the PPC spectra of all individual neurons are averaged. VTA dopamine neurons resonated at higher frequencies and with larger synchrony to regular (green line) than to Poisson stimulation (blue line). Normalizing the frequency axis to the neuronal oscillation frequency of each neuron before averaging (Fig. 4.5D) reiterates our findings of stronger resonance at higher frequencies (1:1 mode, normal-
ized frequency) for regular stimulation (green line) and sub-harmonic resonance to Poisson stimulation (blue line, below half of the normalized frequency, mind the horizontal log axis). The differences between the two stimulation regimes imply that VTA dopamine neurons do not respond linearly to regular and Poisson stimulation.

![Graph A](image)

**Figure 4.5:** Resonance of the VTA dopamine neuron population. **A**, Frequency with the highest synchrony (PPC) for each neuron as a function of its oscillation frequency during regular stimulation (colors indicate the 7 experiments). The neurons resonated strongest with the laser pulses at their oscillation frequency (1:1 mode, black line). **B**, Same as **A** for Poisson stimulation. Neurons resonated primarily on sub-harmonics of their oscillation frequency (1:2 and 1:4). **C**, Mean PPC spectra for all neurons (n = 73) during stimulation (regular (green) and Poisson (blue)). During regular stimulation a peak in resonance was seen around 2 Hz, while the highest resonance was around 1 Hz during Poisson stimulation. **D**, Same data as in **C** but before averaging, the frequency for each neuron was normalized to its oscillation frequency. During regular stimulation (green), the highest synchronization was observed at the oscillation frequency (1:1 mode). During Poisson stimulation (blue) the highest synchronization was observed below half of the normalized frequency (1:2 and 1:4 modes).
4.2.4 Neuron-neuron interactions induced by Poisson stimulation

The dopamine neurons in the VTA population were able to resonate simultaneously to Poisson stimulation, due to its broadband frequency content (Fig. 4.5E), but does resonance to the common Poisson stimulation also induce a structured network state? To this end, the PPC was computed between all pairs of neurons within the recorded population during the stimulation off and on periods. The mean PPC spectrum during stimulation off (Fig. 4.6A) shows no structure in the baseline pairwise neuron interactions. During stimulation on, the PPC spectrum has a broad peak around 1 Hz, indicating neuron-neuron synchrony driven by the Poisson stimulation. The component of the neuronal interactions solely due to common drive by the laser pulses was separated by computing the coherence and the partial coherence (Rosenberg et al., 1989, 1998). The coherence (Fig. 4.6C) confirmed the results of the PPC for neuron-neuron synchrony panel B), although with additional bias (Vinck et al., 2010). The partial coherence indicates that about one-tenth of the coherence between pairs of neurons is not due to common drive by the laser pulses (Fig. 4.6D, mind the different scale of the y-axis). We conclude that the Poisson regime is capable of inducing a network state, mainly due to common drive of the neurons, but one-tenth of the neuron-neuron interactions was not attributable to common drive and represented stimulus state dependent neuron-neuron interactions.

Figure 4.6: Network state induced by Poisson stimulation. A. Baseline PPC spectrum (stimulation off) of pairwise neuron-neuron interactions within the 7 recorded populations. B. Same calculation as in A for stimulation on. The peak around 1 Hz indicates synchrony among pairs of neurons (n = 347). C. Coherence spectrum of neuron-neuron interactions during stimulation on, confirming the results in B. D. Partial coherence spectrum (mind the y-axis scale). Around 10% of the coherence in C indicated local neuron-neuron interactions and was not due to common synchrony.
4.2.5 State switching is fast

The analysis of neuron-neuron synchrony demonstrated that Poisson stimulation induces a network state, which implies state switching. Running correlations between neuronal spikes and the laser pulses per neuron were used to assess the switching speed. During stimulation off we emulated virtual laser events (Fig. 4.7A) with identical time distribution to perform the computation of the running correlation. The transition from stimulation off to on (Fig. 4.7B) and vice-versa (Fig. 4.7C) was aligned at $t = 0$ and then averaged over all recorded neurons. Switching occurred almost instantaneous (~10 s), as our moving window needed a width of 5 s which restricts our time resolution. We conclude that bidirectional switching between the two states induced by Poisson stimulation (on and off) is very fast.

Figure 4.7: Network state transitions. A, Analysis protocol used to calculate state transitions. Virtual spikes with a similar distribution as the actual ones allowed computation of the correlation during stimulation off. B, Mean running correlation between neurons and laser ($n = 73$) during transition ($t = 0$) from stimulation off to on, showing a rapid transition to the correlated state. C, Same as in B for the transition from on to off, showing a rapid transition to the de-correlated state.
4.3 Discussion

The VTA dopamine neuron exhibited spontaneous rhythmic firing with oscillation frequencies between 1 and 5 Hz. Regular 2 Hz laser pulses did not increase the firing rate, but affected the timing of the spikes. This spike timing modulation induced synchrony between the spikes and the external input. The VTA dopamine neurons resonated harmonically to the laser pulse frequency with definite modes (1:1, 1:2, 2:3). Interestingly the 2:3 mode is a rare polyrhythmic mode and hard to measure in biological systems (Pikovsky et al., 2002).

The peak resonance frequency (highest PPC) scaled with the intrinsic oscillation frequency of the neurons. Synchrony was strongest at the intrinsic oscillation frequency, during regular stimulation. In the case of Poisson distributed stimulation the scaling of the resonance frequency was still seen, but neurons resonated most strongly to sub-harmonics of their oscillation frequency. The emphasis on lower modes of resonance (1:2, 1:4) during Poisson stimulation shows that VTA dopamine neurons behave as non-linear filters. They respond differently to narrow (regular) and broad-band (Poisson) input, characterized by altered filter characteristics based on spike timing synchrony. We did not study the cellular mechanisms behind the sub-harmonic resonance to Poisson pulses, which likely involves sub-threshold oscillations (Lampl and Yarom, 1997) as described in several models of the VTA dopamine neuron (Kuznetsov et al., 2006; Medvedev et al., 2003; Wilson and Callaway, 2000). We hypothesize that the observed sub-harmonic resonance can be understood as the stochastic drive of a non-linear oscillator, which has a preferred stimulus phase within the spike cycle (i.e. just before spike threshold) (Tiesinga, 2002; Hata et al., 2010). Poisson stimuli are then less likely to fall within the right time window each spike cycle, than every other cycle or every third or fourth cycle and thus will prefer to push the neuron with sub-harmonics of the oscillation frequency.

Fujisawa and Buzsáki (2011) hypothesized that the VTA is the pacemaker of a 4 Hz local field rhythm, which entrains the prefrontal cortex and the hippocampus (Lisman and Grace, 2005; Fujisawa and Buzsáki, 2011). In-vivo VTA dopamine neuron firing rates are slightly higher (mean ± SD: 5.1 ± 0.6 Hz (Fujisawa and Buzsáki, 2011)) than our in-vitro values, which could relate to the absence of external input or it could indicate that our rhythms are subharmonics of the in-vivo activity. Our data shows that the lateral VTA has self organizing properties, as it can impose its own rhythm on noise-like external input. When stimulated with Poisson pulses, the local VTA network resonated selectively to sub-harmonics of its dominant oscillation frequency. The VTA’s self-organizing frequency-selective output during noise-like input thus represents a mechanism for low-frequency pacemaker activity. Such harmonic coupling between the hippocampus and the VTA in a loop with the prefrontal cortex was also described in vivo (Fujisawa and Buzsáki, 2011) and lead others to speculate that polyrhythmic coupling is a prime mechanism for cross-area interactions (Battaglia and McNaughton, 2011). Our findings of polyrhythmic resonance modes and selective frequency filtering, provide a mechanism by which the VTA can lock to input and drive other brain areas.

The broad-band nature of Poisson stimulation allowed us to drive simultaneously recorded neurons effectively, as each neuron can resonate to its preferred frequency. This common drive induced neuron-neuron synchrony (pairwise PPC), which was closely centered around the sub-harmonic 1 Hz of the mean oscillation frequency (2 Hz). Such noise-induced-synchrony has rarely been recorded in a neuronal system and is a recent addition to our understanding of
the brains’ function in the presence of noise (Hata et al., 2010; Ermentrout et al., 2008; Galán et al., 2006). Partial coherence analysis showed state-dependent (stimulus on and off) neuron-neuron interactions (10%). These neuron-neuron interactions are an emergent property of the Poisson stimulus induced network state, which was able to switch almost instantaneously between stimulation on and off.

Our findings have broad functional implications. The VTA has long been implicated in stimulus-reward coupling, with reward prediction based on an internal time code. However, the neural substrate of such a time code in the range of seconds is as yet unknown (Schultz, 1997; Lak et al., 2016). 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. 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 and as a reward prediction error code in general.

Our research indicates that the VTA network is a non-linear filter bank when responding to external input, which can decode timing information related to stimulus-reward processing and generate fast switchable internal network states. These network states are self-organizing as they impose structure on noisy input and could underly a pacemaker role of the VTA, linked to entrainment of the hippocampus and the prefrontal cortex during cognitive tasks.