Drumming with dopamine neurons
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CHAPTER 3

Tuning of neuronal interactions in the lateral Ventral Tegmental Area by dopamine sensitivity

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Abstract

The ventral tegmental area (VTA) contains a considerable population of rhythmically firing dopaminergic neurons, which are influenced by auto-inhibition due to extra-synaptic dopamine release resulting in volume transmission. Using a Multi-Electrode-Array we simultaneously recorded from multiple VTA dopamine neurons and studied their mutual interactions. We observed that the dopamine sensitivity (EC50) of the neurons (i.e. the relation between dopamine concentration and firing rate) was quite variable within the recorded population. The interactions between pairs of neurons were quantified using Granger causality. We found that the dopamine sensitivity determined the role of a neuron in local VTA network. Highly sensitive neurons became followers (of the population rhythm), whereas less sensitive dopamine neurons played a more leading role. This was confirmed by the application of sulpiride which reduces the dopamine sensitivity of all neurons through competition and abolished the structure in the interactions. These findings imply that therapeutics, which have an easy to understand effect on firing rate, could have a more complicated effect on the local VTA network organization, through volume transmission principles.
3.0 Introduction

The Ventral Tegmental Area (VTA) is one of the dopamine nuclei in the midbrain, positioned alongside the Substantia Nigra and Red Nucleus. Dopamine neurons are the most abundant neuron type in the VTA (Nair-Roberts et al., 2008). They share direct synaptic connections but are also connected through local inter-neurons (Bayer and Pickel, 1990; Omelchenko and Sesack, 2009). Dopamine neurons in the midbrain come in different varieties (Liss and Roeper, 2008), related to their region of expression and projection areas (Lammel et al., 2008). Dopamine neurons not only release dopamine, but they are also sensitive to dopamine as they contain several types of dopamine receptors. The sensitivity to dopamine of the dopamine neurons varies over these different sub-divisions (Lammel et al., 2008; Margolis et al., 2008). In this study we recorded from the lateral VTA mainly containing mesolimbic projecting dopamine neurons with classic dopamine neuron phenotypes (Lammel et al., 2008).

VTA dopamine neurons generate action potentials (spikes) and they can exhibit spontaneous activity at a low firing rate (1-5 Hz) in vivo as well as ex vivo (Werkman et al., 2001; Bayer et al., 2007). The spontaneous rhythmic neuronal firing activity of the dopamine neurons is important for baseline dopamine levels in the downstream projection areas of the VTA (Kalivas, 1993). Within the VTA the spike activity also leads to dopamine release into the extracellular space through calcium dependent axonal and somatodendritic release (Ford et al., 2010; Rice et al., 2011). The activity-modulated extracellular dopamine levels within the VTA reach values that are sufficient to reduce the firing of dopamine neurons, through activation of the D2-receptor-coupled GIRK channels (Slaney et al., 2013; Ford et al., 2010; Cragg et al., 2001). The released dopamine induces auto-inhibition in the releasing neurons but more importantly it also diffuses far enough to produce a population signal that can inhibit other dopamine sensitive neurons (cross-inhibition) (Cragg et al., 2001; Zoli et al., 1998). The combination of auto- and cross-inhibition represents an intricate feedback mechanism that facilitates local network interactions. Communication through extra-synaptic neurotransmitter concentrations is known as volume transmission (Cragg et al., 2001; Zoli et al., 1998; Zoli and Agnati, 1996) and the dopamine system is the classic model system. Communication between pairs of neurons via dopamine release and the modulation of Ih currents has been observed in the Substantia Nigra (Vandecasteele et al., 2008), but in the VTA direct evidence of volume transmission is scarce.

In this study we reason that if volume transmission in the VTA is combined with different sensitivities for dopamine, the functional role of individual dopamine neurons in the VTA should be distinct. Neurons with a high dopamine sensitivity could be forced to synchronize with the dopamine signal, in contrast to insensitive neurons. In order to investigate this hypothesis we need to record simultaneously from a relatively large population of spiking dopamine neurons, whose dopamine sensitivity needs to be established individually. The 60 channel Multi-Electrode-Array (MEA) is an ideal recording tool for spiking neurons in a brain slice. The MEA provides good visual control over the recording area and the acute brain slice allowed stable pharmacological recording conditions.

In this study we define the dopamine sensitivity from the relation between the observed firing rate and the applied concentration of the dopamine agonist quinpirole (EC50). The EC50 is a much wider definition than the classic IC50 affinity of the D2-receptor for the drug (Neubig et al., 2003). The dopamine sensitivity, besides the affinity of the receptor,
also includes all steps in the signaling cascade that couple the receptor via a g-protein to a membrane conductance that finally modulates the neuronal firing rate (Ford, 2014; Lüscher and Slesinger, 2010).

The main goal of this paper is to relate the role a neuron plays in the local VTA network to its dopamine sensitivity (EC50), in order to reveal a distinct organization in the dopamine neuron population. To this end, the combined activity of the dopamine neuron population was taken as an approximation of the extracellular activity-dependent dopamine signal experienced by each individual neuron. Interactions between one dopamine neurons’ spike activity and the combined spike activity of all other neurons in the recorded population were analyzed. The directional interaction between a neurons’ spike activity and that of the rest of the population was computed using Granger causality (Granger, 1969; Ding et al., 2006). Granger causality measures the predictive value of a neurons’ spike activity for the spike activity of the rest of the population and vice versa; from here on we will call this parameter the ‘influence’. Influence can have two directions: the influence of the population on the individual neuron is defined as ‘inward’, while ‘outward’ influence is exerted by an individual neuron on the population (illustration, Fig. 3.4). We find that dopamine sensitivity is heterogeneous property within the lateral VTA and organizes the network interactions between VTA dopamine neurons, by assigning leading and following network roles.
3.1 Methods

3.1.1 Slice preparation

Male wistar rats (Harlan, Zeist, The Netherlands) between 75 and 100 gram were decapitated. The midbrain was dissected and kept in artificial cerebral spinal fluid (ACSF) at 4 °C, containing (in mm) NaHCO$_3$ 25, D-glucose 10, CaCl$_2$ 2.5, NaH$_2$PO$_4$ 1.25, MgSO$_4$ 1.3, KCl 3.5, NaCl 120, which was bubbled with carbogen (95 % O$_2$; 5 % CO$_2$), pH was 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. Two to three slices containing the medial to caudal 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. All experiments and methods were approved by the ethical committee for animal experimentation of the University of Amsterdam.

3.1.2 Electrophysiology

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 midbrain 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). A 20 minute acclimatization time preceded the recordings.

3.1.3 Data acquisition

The extracellular recordings with the 60-channel MEA 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. Voltage peaks (positive and negative) were detected, with a relatively low threshold to prevent detection failures. The signal around each peak (± 3 ms) was extracted and K-means clustering was used to cluster the largest two principle components and the maximum amplitudes of the peak waveforms. The auto-correlation and inter-spike-interval distribution of the peaks in the various 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 the cluster with the largest peak amplitude.

3.1.4 Spike train analysis

The oscillation frequency is a measure for the preferred intrinsic rhythm of the neuron. It was computed as the inverse of the mean interval between peaks (side-lobes) in the auto-correlation (Fig. 3.2A), including the interval between the 0-lag and the first side-lobe. The irregularity of neuronal firing in the VTA was assessed using a measure of local variation (LV) defined in (Shinomoto et al., 2005), which measures the similarity between consecutive
inter spike intervals (ISIs). This metric ranges from 0 (perfectly regular firing) to 1 (Poisson distributed firing) and above 1 for burst-like firing (Shinomoto et al., 2009).

\[
LV = \frac{3}{n-1} \sum_{i=1}^{n-1} \left( \frac{ISI_i - ISI_{i+1}}{ISI_i + ISI_{i+1}} \right)^2
\] (3.1)

The population output of a VTA dopamine neuron population was investigated as a spike-based signal comparable to the VTA’s local field signals recorded by other researchers (Fujiisawa and Buzsáki, 2011). Neurons contributed equally to the population output, by normalizing the point processes (spike trains binned using a 30 ms bin size) to the total number of spikes they contained before summing them for all neurons in a recorded (slice) population. The population output was convoluted with a Gaussian kernel (SD: 60 ms) to convert it into a spike density function. The power spectral density of this spike density function was computed, to assess the fluctuations in the population output, using Welch’s method (Welch, 1967; Hunter, 2007).

### 3.1.5 Granger causality

Granger causality (Granger, 1969) was computed by fitting a VAR(2) model (Brett et al., 2009) to the spike train of a neuron and to the combined spike train of all other neurons (duration \(\geq 10\) min). The VAR(2) model estimates the directional predictions between two processes. Granger causality was computed in the frequency domain from the VAR(2) fit as described in (Ding et al., 2006). Taking the neuron as a reference we define the outward Granger causality as the influence of the neuron on the rest of the population and the inward Granger causality as the influence of the population on the neuron under investigation (illustration Fig. 3.4). Before fitting each spike train was binned, sampled at 100 Hz and the mean value was subtracted to remove the constant offset. The combined population spike train counted the number of spikes in the bins, while the individual neuron’s spike train was binarized. Two spike cycles, based on the oscillation frequency of the neuron, determined the number of lags of the VAR(2) model (e.g.: 1 Hz oscillation frequency, 1 s cycle time, two times 1 s is fitted, thus 200 lags of 10 ms). In essence Granger causality predicts spike timing based on the information in the given channel and as such it could well be sensitive to cycle skipping in an otherwise regular firing pattern. To study the effect of spike cycle skipping on the Granger causality, we fitted the VAR(2) model twice, once for each direction. Each time we filled up the skipped spikes in the process on which the Granger influence was analyzed. This was done by interpolating spikes within the gaps, based on the oscillation frequency of the neuron. For the combined population spike train, missing spikes of individual neurons were inserted before summing the spike trains.

### 3.1.6 Solutions

Quinpirole (quinpirole hydrochloride, Sigma-Aldrich) 1 mM and 10 mM stock solutions were made in H₂O. Sulpiride (Sigma-Aldrich) 1 mM stock solution was made in 0.001 N HCl. Stock solutions were stored at −20 °C and warmed up to room temperature just before use.
3.1.7 Experimental conditions

Each experiment consisted of two cumulative administrations of the dopamine agonist quinpirole, each preceded by a long-term measurement of network activity (Fig. 3.1). The baseline network measurement, which lasted 20-30 min, was followed by the first cumulative quinpirole concentration series (0.03, 0.1, 0.3 µM). The second network measurement (10-20 min) and following cumulative quinpirole concentration series (0.3, 1, 3, 10 µM) were performed in the presence of 0.1 µM sulpiride (Fig. 3.1).

<table>
<thead>
<tr>
<th>First</th>
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<tr>
<td>Network activity</td>
<td>quinpirole</td>
</tr>
<tr>
<td>measurement</td>
<td>EC50 assessment</td>
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<tr>
<td>start 20-30 min</td>
<td>10-20 min</td>
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<td>7 min per concentration</td>
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Figure 3.1: experimental protocol to assess the EC50 of quinpirole and measure population activity in the absence (baseline) and presence of 0.1 µM sulpiride.

3.1.8 Pharmacological analysis

The neuronal firing rate was computed from five second long spike train segments and averaged over the last three minutes of each quinpirole administration. The EC50 for quinpirole was assessed by fitting a concentration response curve (Hill curve) to the normalized neuronal firing rate of each individual neuron in response to the various concentrations (c). For the individual fits the Hill coefficient (h) was fixed based on fit of the joint response per slice (mean value for h over the slices h = 1.2 ± 0.2, n = 4). The relation between agonist (quinpirole) concentration and the resulting reduction in neuronal firing rate was then described by the ’dopamine sensitivity’ (EC50). Although the equation is the same, the EC50 metric is different from the classical IC50 for receptor binding. The EC50 incorporates receptor affinity, but also all other intracellular mechanisms that relate receptor activation to neuronal firing (Neubig et al., 2003). The ’dopamine sensitivity’ (EC50) of our neuron for quinpirole is the concentration at which the neuronal firing rate is 50 % of the baseline rate.

\[
\text{fractional response}(c) = 100 \times \frac{EC50^h}{(c^h + EC50^h)} \tag{3.2}
\]

Application of the antagonist sulpiride strongly suppresses the firing modulation by quinpirole or (endogenous) dopamine. The shift in the EC50 for quinpirole due to the presence of the antagonist sulpiride (c_{sulpiride} = 0.1 µM) was quantified by computing pA₂ value, which was computed from the EC50 in the absence (1) and presence (2) of sulpiride as:

\[
pA_2 = \log_{10}\left(\frac{EC50_2}{EC50_1} \frac{EC50_1}{c_{sulpiride}} - 1\right) \tag{3.3}
\]
3.2 Results

3.2.1 Spontaneous population activity

In total 44 VTA dopamine neurons were recorded in the 4 experiments. VTA dopamine neurons have a well defined intrinsic rhythm. Fig. 3.2A shows this rhythm as peaks in the auto-correlation of a typical spike train of a single spontaneously active VTA neuron. This rhythm was also present in the pooled spike train output of the populations, and then appeared as peak in the power spectrum calculated from the combined spike train (Fig. 3.2B). The irregularity of the intrinsic rhythm was quantified using the Local Variation (LV, Eq. 3.1). The LV distribution was unimodal (Hartigan’s dip-test, $p = 0.28$, Fig. 3.2C) and no differences were found between slices (ANOVA, $p = 0.95$). VTA dopamine neurons showed substantial variation in firing irregularity (mean ± SD: 0.25 ± 0.19). This rhythmic activity on the level of the neuron and population were used for further network analysis.

![Figure 3.2](image)

Figure 3.2: Rhythmic activity on the neuron and population level. A, Auto-correlation of the rhythmic firing of a dopamine neuron with high regularity. B, Power spectrum of the pooled spike trains of 14 neurons in one experiment, showing preferred oscillations in the spike activity. C, The firing irregularity (LV) of the recorded VTA dopamine neurons was quite variable and followed a unimodal distribution.

3.2.2 Dopamine sensitivity

The assessment of the dopamine sensitivity (EC50) was performed after each of the two network activity measurements (Fig. 3.1). All neurons responded to the cumulative quinpirole application with a cessation of firing. 37 of the 44 neurons exhibited a monotonic response to the first series of cumulative quinpirole concentrations and allowed a baseline EC50 to be assessed by fitting a concentration-response curve (Fig. 3.3A). The distribution of the baseline EC50 values for quinpirole (Fig. 3.3, indicates substantial variation within the population of VTA dopamine neurons (mean ± SD: 66 ± 52 nm). An analysis of variance of the EC50 values for quinpirole demonstrated that the variance was inherent to the neuronal population within the lateral VTA and not related to the different experiments (slices) (Kruskal-Wallis, $p = 0.65$). These results lead us to further investigate the functional consequences of the large variation in dopamine sensitivity (EC50) i.e. the coupling between D2-receptor activation and the inhibition of neuronal firing rate in VTA dopamine neurons.
Figure 3.3: Heterogeneous EC50 for quinpirole across VTA dopamine neurons. A. Typical concentration response curve for quinpirole on firing rate of a single VTA neuron, demonstrating cessation of firing at higher concentrations. Solid line is the fit (Eq. 3.2) using the population mean value for \( h = 1.2 \). B. Distribution of quinpirole EC50 values for all neurons with a monotonic concentration response (\( n = 37 \)) pooled from all four experiments.

3.2.3 A neurons’ role in the network

The functional interactions within the local VTA population were further investigated, as we hypothesized a relation between them and the heterogeneous dopamine sensitivity, based on the volume transmission principle. The role a dopamine neuron played within the local VTA network was assessed in relation to the population activity (combined spike train) and was quantified using Granger causality (Granger, 1969), which measures the directional influence between a neurons’ spike train and the population signal during a relatively long (> 10 min) period of recorded network activity.

In terms of Granger causality, we define outward’ influence from the neuron on the population and ‘inward’ influence from the population on the neuron (Fig. 3.4, inset). The two distinct roles a neuron can play in the network were illustrated by neuron A and B (Fig. 3.4).

The power spectra, derived from the VAR(2) model fit, show that neuron A oscillated at a relatively low frequency (±0.5 Hz, Fig. 3.4A1), which was about half the frequency of the most dominant oscillation in the population output (Fig. 3.4A3). In the inward Granger spectrum a large peak was seen at the oscillation frequency of the population (Fig. 3.4A4: inward i.e. population to neuron), while such a peak was absent in the outward Granger spectrum (Fig. 3.4A2: outward i.e. neuron to population). This indicated that this neuron with an EC50 of 15 nM received more influence than it exerted.

The reverse can be observed in the second sample neuron B which oscillated at approximately the same frequency as the population (Fig. 3.4B1 and 3: compare peaks in neuron and population). In the outward Granger spectrum a prominent peak was seen (Fig. 3.4B2: outward i.e. from neuron to population), while such a peak was absent in the inward Granger spectrum (Fig. 3.4B4: inward i.e. from population to neuron). In contrast to neuron A, neuron B with an EC50 of 150 nM exerted more influence than it received. These two neurons illustrate the two distinct roles a neuron can play within the network, which can be interpreted as either following (neuron A) or leading (neuron B). This characterization of the local VTA network interactions allowed us to study their relation to the dopamine sensitivity.
Figure 3.4: The roles a neuron can play within the local VTA network. Illustration, inward and outward influence is defined between the spike train of the individual neuron and the combined spike activity of all other neurons in the recorded population. A, example neuron with an EC50 of 15 nm. The power spectra showed regularly interspersed peaks for both the neuron (A1) and the population (A3), indicating rhythmic activity. The neurons’ activity oscillated at about half the frequency of that of the population. The Granger spectra showed a peak in the inward direction (1 Hz, A4), which coincided with the dominant peak in the population output spectrum (A3 population). A similar peak was absent in the outward Granger spectrum. B, example neuron with an EC50 of 150 nm. The power spectra showed that the neuron (B1) and population activity (B3) oscillated with a similar dominant frequency (1 Hz). The Granger causality spectra showed a large peak in the outward direction (1 Hz, B2), which coincided with the dominant peak of the neurons’ population power spectrum (B1). A similar peak was absent in the inward Granger spectrum.
3.2.4 Network organization through volume transmission

VTA dopamine neurons less sensitive to dopamine were hypothesized to take a more leading role, due to them contributing to the extracellular dopamine signal, while being less inhibited by it. Fig. 3.5A shows the baseline inward and outward Granger for all the recorded dopamine neurons \((n = 44)\), at either the most prominent oscillation frequency of the neuron (outward) or that of the population (inward). The role a neuron played in the network was defined as the difference between the inward and outward Granger. To investigate the relation between the EC50 of a neuron and its role in the network, the 34 neurons of which both a baseline EC50 and Granger causality could be assessed were analyzed further. The relative role a neuron played correlated with the EC50 of that neuron. Less dopamine sensitive neurons exerted more outward influence than the received inward influence (Fig. 3.5B, Pearson: \(\rho = 0.44, p = 0.01, R^2 = 0.19\)). The spike insertion used to eliminate the influence of spike skipping (see methods) preserved the correlation between the EC50 and the network role of the neuron (Fig. 3.5C, Pearson: \(\rho = 0.51, p = 0.002, R^2 = 0.26, n = 34\)). We also performed a multi-linear regression of the EC50 and the firing irregularity (LV) of a neuron on its relative network role (compensated for spike cycle skipping). The EC50 for quinpirole \((\beta = 0.008, p = 0.002, R^2 = 0.33, n = 34)\), but not the LV \((\beta = -0.007, p = 0.076)\) correlated with the net network role. These results indicate that the dopamine sensitivity of a VTA dopamine neuron related to the role it plays in the local VTA network, with less sensitive neurons taking a more leading role.

![Figure 3.5](image_url)

Figure 3.5: The relation between dopamine sensitivity (EC50) of a neuron and its role in the VTA network. A, inward versus outward influence (Granger) for all neurons recorded during baseline. The variation indicates some neurons exert more influence than they receive and vice versa. B, outward minus inward influence versus the EC50 for quinpirole for all neurons of which both could be assessed \((n = 34\) over 4 experiments indicated with colors). The EC50 and the relative role in the network correlated positively. C, same as B, but in this case spike skipping was controlled for \((n = 34\) over 4 experiments indicated with colors). The quinpirole EC50 and the relative role in the network correlated positively.
### 3.2.5 Reducing dopamine sensitivity eliminates the network structure

If a neuron's role in the network is causally related to its EC50, then manipulating its dopamine sensitivity should affect it. The dopamine D2 receptor antagonist sulpiride (0.1 µm) was used to compete with quinpirole for dopamine D2 receptor binding. In the presence of 0.3 µm quinpirole, which had suppressed spike firing activity, the addition of sulpiride restored the activity of 36 out of the original 44 neurons, whereafter the EC50 in the presence of sulpiride could be determined using cumulative quinpirole administrations (Fig. 3.1 and 3.6A). Fig. 3.6B shows the distribution of the EC50 values in the presence of sulpiride for the 13 neurons, which exhibited a monotonic concentration response (mean ± SD: 4.1 ± 2.1 µm). Sulpiride induced a shift in the EC50 value, which was quantified by the pA2 (Eq. 3.3) in all neurons that had a monotonic response to both cumulative quinpirole administrations (mean ± SD: 8.8 ± 0.27 µm, n = 10). This indicated that application of 0.1 µm sulpiride made the VTA dopamine neurons about two orders of magnitude less sensitive to dopamine. We investigated whether this reduction in dopamine sensitivity in the presence of sulpiride had a consequence for the observed network interactions. In the presence of 0.1 µm sulpiride the correlation between the baseline EC50 and the net Granger causality seen in Fig. 3.5C was no longer observed (Fig. 3.6C, pearson: $\rho = -0.17$, $p = 0.4$, $R^2 = 0.027$, $n = 27$). In addition, when we directly compared the two correlations in the absence and presence of sulpiride, we concluded that they were significantly different (Fisher’s Z-test, $p < 0.05$). These results show that reducing the dopamine sensitivity abolishes the functional network structure that we ascribed to it. This strongly confirms that the heterogeneous dopamine sensitivity defines roles of the VTA dopamine neurons, by modulating their interaction with the extracellular dopamine signal in the network.

![Figure 3.6](image-url) **Figure 3.6:** Sulpiride abolishes the relation between the dopamine sensitivity and the network structure. **A.** Concentration response curves of the firing rate of a neuron in the absence and presence of 0.1 µm sulpiride. The shift between the two response curves was due to agonist-antagonist competition. **B.** Distribution of the EC50 for quinpirole in the presence of 0.1 µm sulpiride for all recorded of which the second concentration-response curve could be quantified (n = 13 over 4 experiments). **C.** Outward minus inward influence in the presence of 0.1 µm sulpiride versus the EC50 (baseline) for all neurons of which both could be assessed (n = 27 over 4 experiments indicated with colors). The previously seen correlation between the EC50 and the relative role in the network (outward minus inward granger) was absent.
3.3 Discussion

The spontaneously active dopamine neurons in the VTA release dopamine into the extracellular space. This release is somadendritic and depends on their coordinated activity (Ford et al., 2010; Rice et al., 2011). In the VTA, extracellular dopamine activates D2-receptors on the neuron itself as well as on surrounding dopamine neurons. Through a G-protein the receptors couple to GIRK channels that hyperpolarize the membrane and reduce neuronal firing rate (Ford et al., 2010; Cragg et al., 2001). This population phenomenon is known as volume transmission (Zoli et al., 1998).

Here we assessed the dopamine sensitivity (EC50) of individual spontaneously active dopamine neurons. The EC50 definition includes the classical receptor affinity, but also all other steps mentioned above that link the extracellular dopamine concentration to neuronal firing rate. The coupling from receptor binding to firing rate includes several steps. Starting at the GIRK signal transduction pathway and ending with the mechanisms held responsible for the membrane oscillation and spike generation. These mechanisms consist of voltage dependent, ion-selective membrane conductances with a prominent role for either calcium currents and/or persistent sodium currents (Khaliq and Bean, 2010; Drion et al., 2011).

The dopamine sensitivity was shown to be a heterogeneous property of the VTA dopamine neurons in the lateral VTA, which consists mostly of mesolimbic projecting neurons with the classic dopamine neuron phenotype (Lammel et al., 2008). Our statistical analysis revealed that the observed variation in EC50 was intrinsic to the neurons and not due to experimental variables such as the slicing. Dopamine sensitivity ranged from highly sensitive (EC50 of around 20 nm) to less sensitive (EC50 of higher than 100 nm). We hypothesized that the dopamine sensitivity should be an important factor for how each individual neuron couples to the volume transmission signal and set out to reveal the functional consequences.

The relation between each neuron and the population was determined using Granger causality, a statistical method that establishes the directional interaction between a neurons’ spike activity and the combined activity of the surrounding population (Granger, 1969; Ding et al., 2006). We defined an ‘outward’ influence of each neurons’ activity on the rest of the population and an ‘inward’ influence of the population activity on each neurons’ activity. VTA dopamine neurons less sensitive to dopamine had a net outward influence i.e. take a more leading role in the local network, by influencing the spike activity of the surrounding neurons more strongly than being influenced by them. For more dopamine sensitive neurons the inverse picture emerged. Our explanation based on the volume transmission principle was further substantiated by rendering the neurons relative insensitive to the volume transmission signal, through the application of a competitive dopamine D2-receptor antagonist (sulpiride). Sulpiride reduced the dopamine sensitivity of all neurons by two orders of magnitude. Re-assessing the network interactions during administration of sulpiride, confirmed that the correlation between the dopamine sensitivity of a neuron and its role in the network was now abolished.

Our results suggest that extracellular dopamine signals, through volume transmission, not only directly feed back on the firing rate of neurons in the local VTA circuit, but due to the differences in sensitivity also organize a spatial and hierarchical structure in the VTA interactions and output pattern. Thus sending additional spatially coded information to the projection areas. The VTA is involved in many cognitive activities (Schultz, 1998) and the dopamine-GIRK signaling pathway forms an important therapeutic target for psychi-
atriac diseases (Beaulieu et al., 2015). The multitude of signaling components involved in the dopamine signal transduction system makes it hard to predict the therapeutic outcome of even very specific drugs (Liperoti et al., 2008).

The consequences of the volume transmission related mechanism that we unveil here imply the presence of an additional emergent property in the VTA output, spatial organization, that responds to dopaminergic drugs. This response is not easily predicted, because it cannot be directly related to the modulation of the firing rate of individual dopamine neurons. Volume transmission in combination with large variation in dopamine sensitivity thus mediates emergent network level effects of any pharmacological substance, which acts on the D2-receptor. Studying these pharmacological effects on the network level, will ultimately deepen the understanding of drug (side-) effects of for instance anti-psychotics.