MULTI ELECTRODE ARRAY

Brain nuclei

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MAIN SUMMARY

- ❑ Habenular nucleus
- ❑ Ventral tegmental area
- ❑ Substantia nigra
- ❑ Subthalamic nucleus
- ❑ Periaqueductal grey matter
- ❑ Thalamic Reticular Nucleus

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HABENULAR NUCLEUS

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SUMMARY - Habenular nucleus

Materials & Methods

- Information about the Habenula
- Materials & Methods

Results

- Non competitive NMDA receptor antagonist – **ketamine**

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INTRODUCTION - Habenular nucleus

- Depression has been suggested to be the result of maladaptive changes in specific brain circuits. Recently, the lateral habenula (LHb) has emerged as a key brain region in the pathophysiology of depression.

- Increasing evidence from rodent, non-human primate and human studies indicates that the aberrant activity of the LHb is associated with depressive symptoms such as helplessness, lack of pleasure (anhedonia), and excessive negative focus.

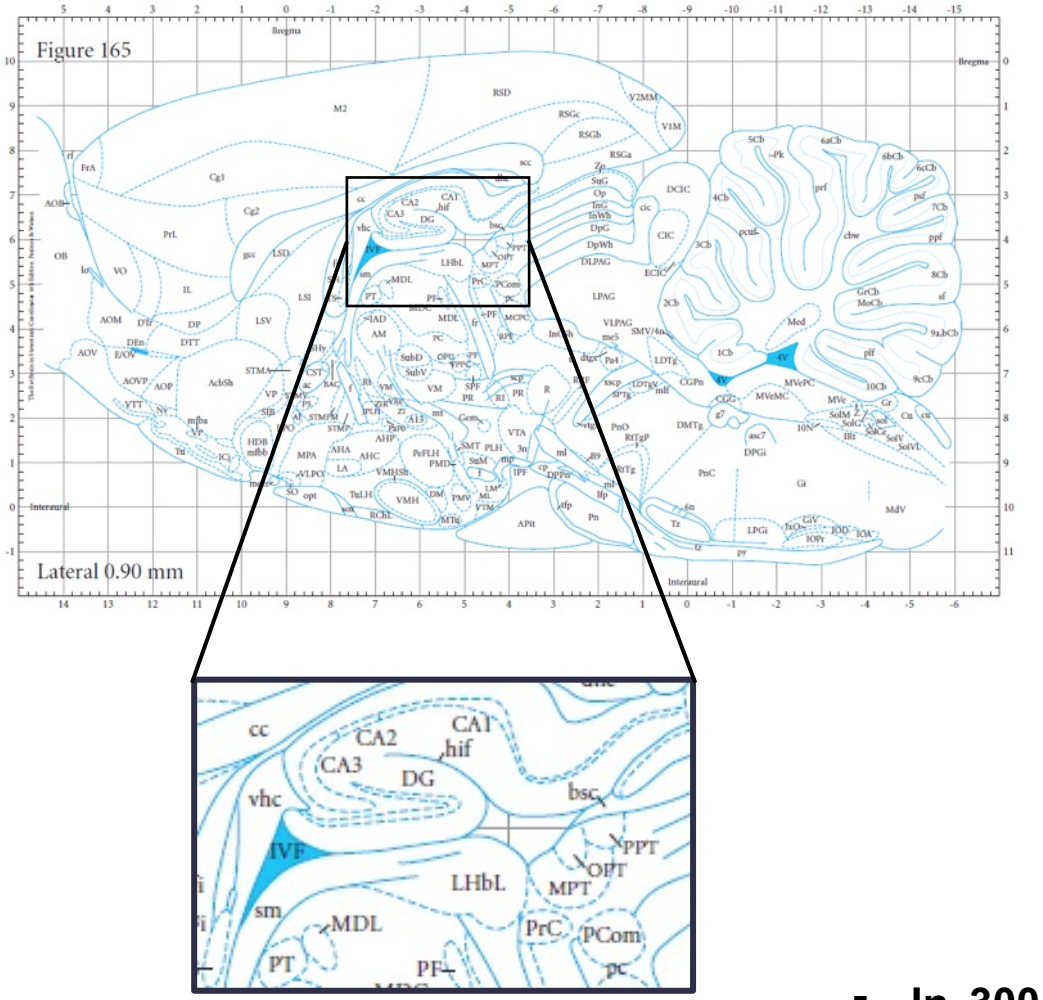
- Circuitry-wise, the LHb acts as a relay station that interconnects the limbic forebrain with depression-related monoaminergic centers including the ventral tegmental area (VTA) and raphe.

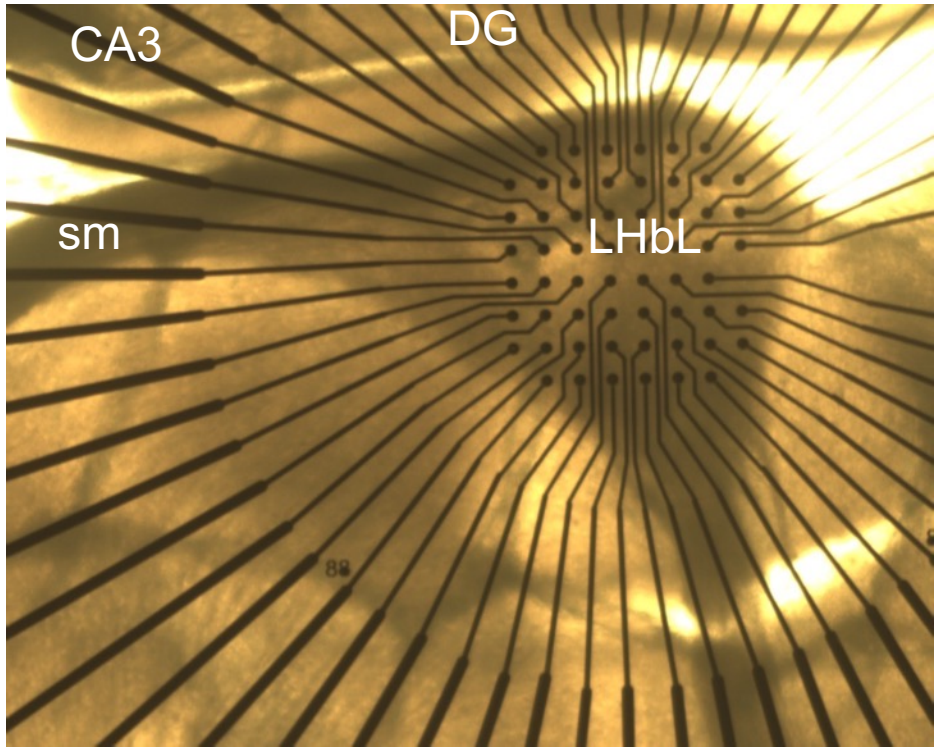
Lateral habenula in the pathophysiology of depression | Elsevier Enhanced Reader

MATERIALS & METHODS - Habenular nucleus

Main summary
Habenula summary

Lateral habenular nucleus – lateral part (LHbL) – Sagittal slices





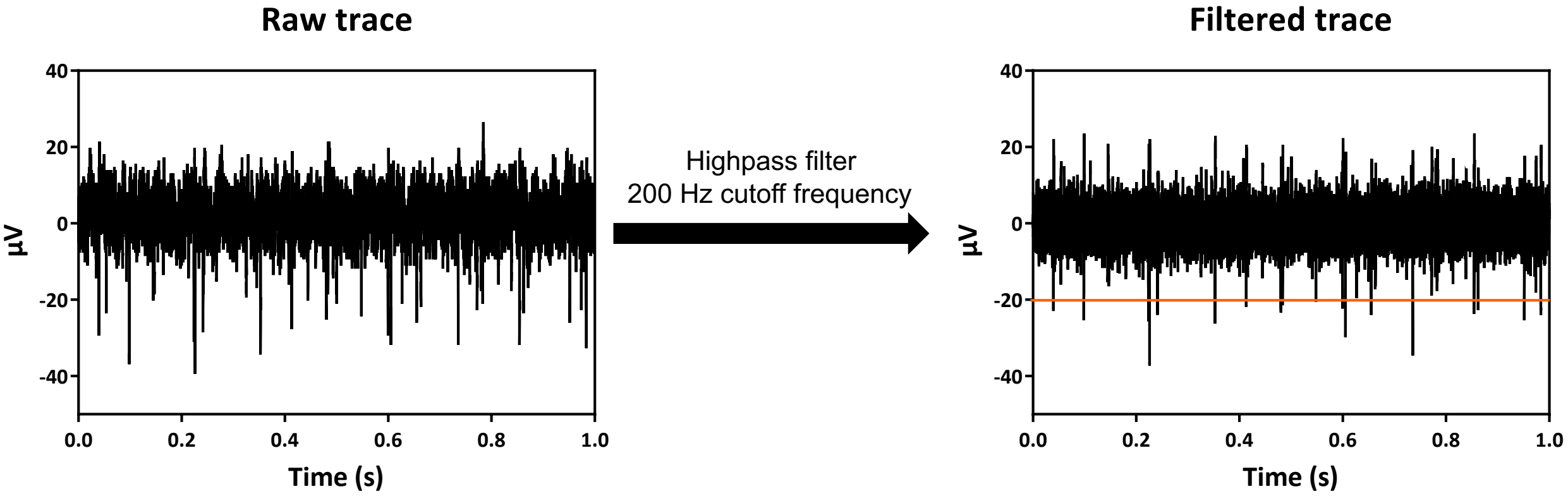
DG: Dentate gyrus
LHbL: Lateral habenular nucleus, lateral
sm: stria medullaris thalamus

- **In 300 µm thick sagittal slices**, lateral habenula is a well delineated nucleus that MEA electrodes (spaced by 100 µm) appropriately cover.

MATERIALS & METHODS - Habenular nucleus

Main summary
Habenula summary

Firing analysis & validation criteria



- Action potentials (APs) amplitude have to be higher than the threshold (-20 µV or -4 SD) to be counted.
- After a 10-minute period of anoxia, firing activity must be abolished.
- Data are binned by 30 s slots and presented as a function of time (± SEM).

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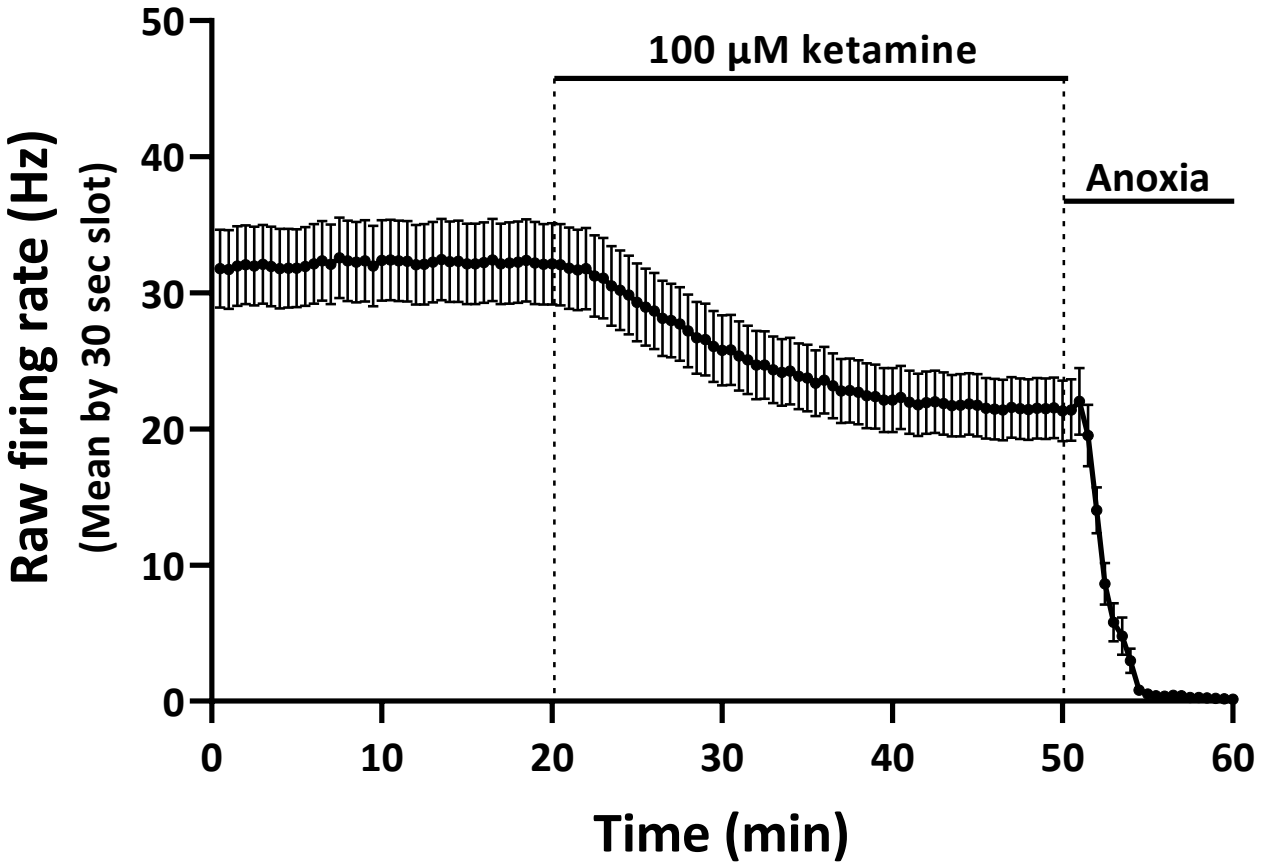
RESULTS - Habenular nucleus

Main summary
Habenula summary

NMDA receptor

Ketamine



100 µM ketamine (104 electrodes, 4 slices from 2 rats)

"LHb neurons show a significant increase in firing activity in depressive-like animals, which is reversed by ketamine." (Yang al. - Nature 2018).

"Blockade of NMDAR-dependent firing activity in the 'anti-reward center', the lateral habenula (LHb), mediates the rapid antidepressant actions of ketamine in rat and mouse models of depression." (Yang al. - Nature 2018).

- Habenula neurons display a sustained and steady firing rate.
- 100 µM ketamine, a non competive NMDA receptors antagonist, substantially reduced the firing rate of habenula neurons.
- Habenula neurons are very sensitive to anoxia. The firing activity was completely abolished after 5 minutes of oxygen deprivation.

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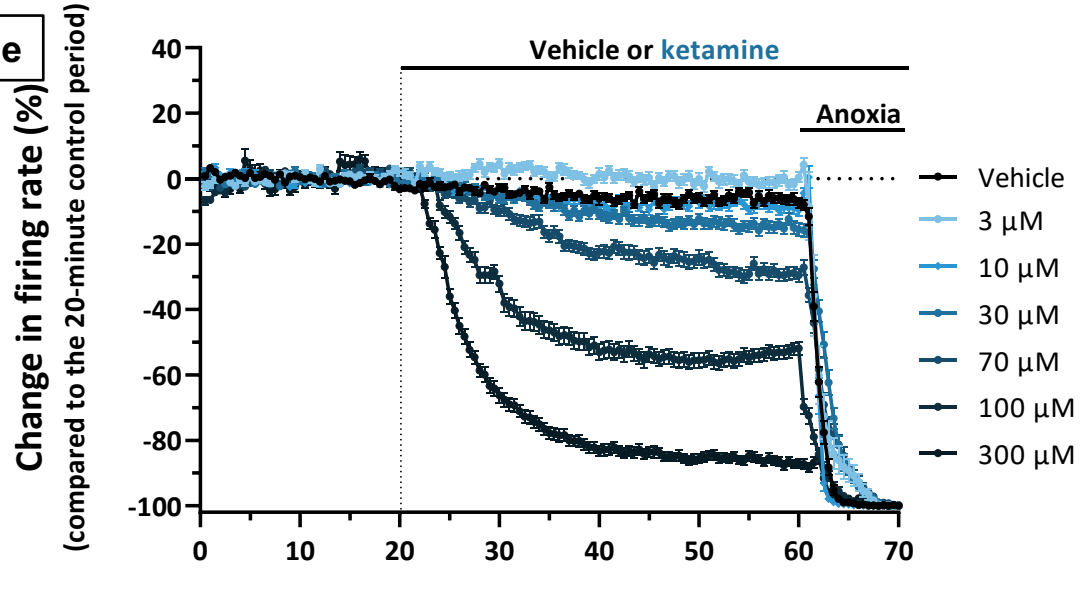
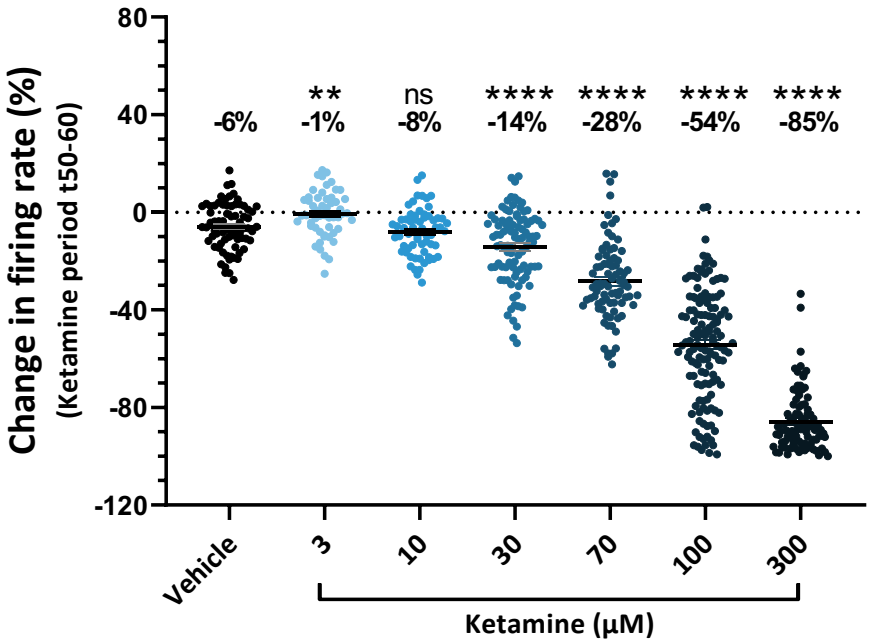
RESULTS - Habenular nucleus

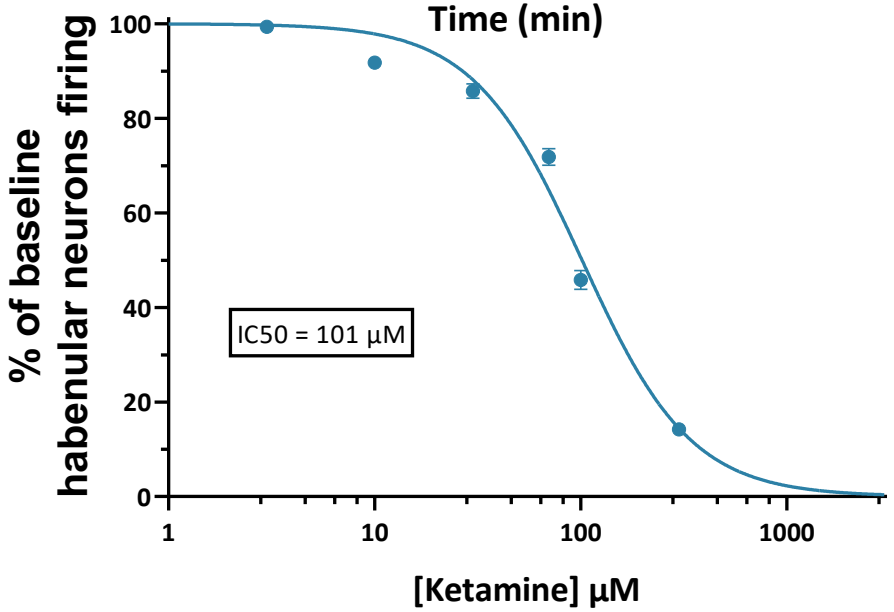
Main summary
Habenula summary

NMDA receptor

Ketamine







- Ketamine dose-dependently decreased the firing activity of habenular neurons with an IC_{50} close to 100 µM.
- The advantage of recordings within the habenula is that the large number of electrodes displaying activity within each slice (~15-20) allows to overcome the variability inherent to biological systems and to get robust results from a restricted number of slices.

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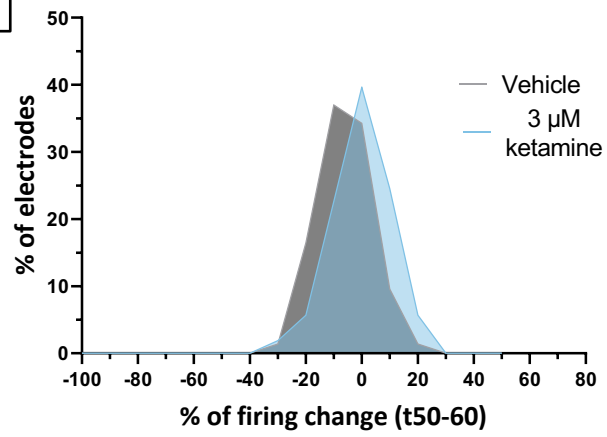
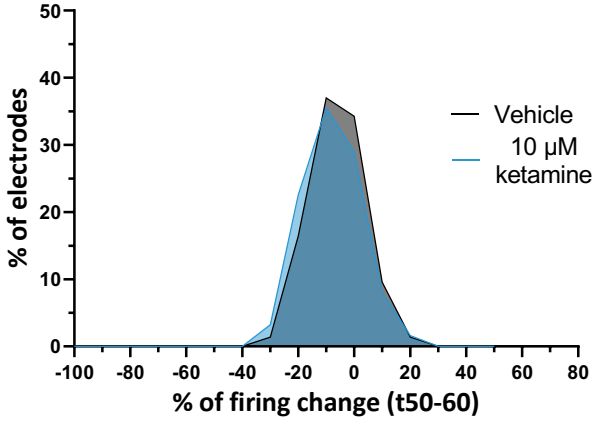
RESULTS - Habenular nucleus

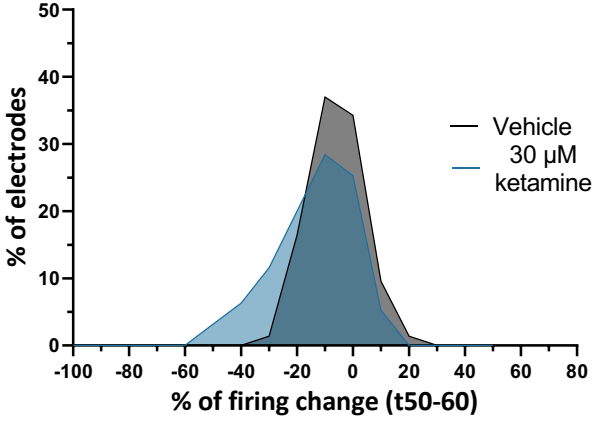
Main summary
Habenula summary

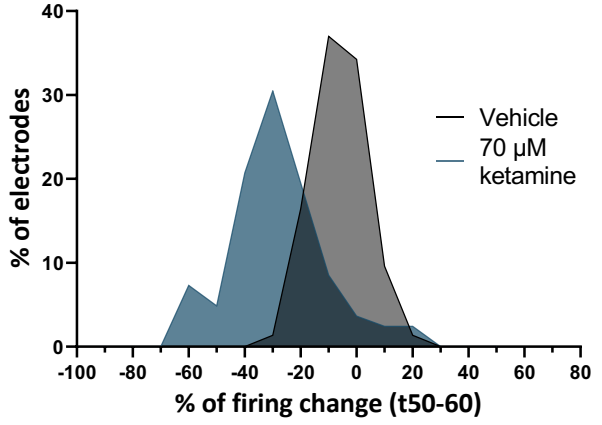
NMDA receptor

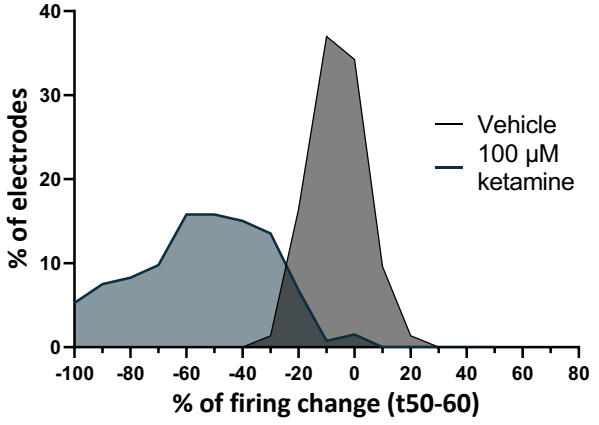
Ketamine

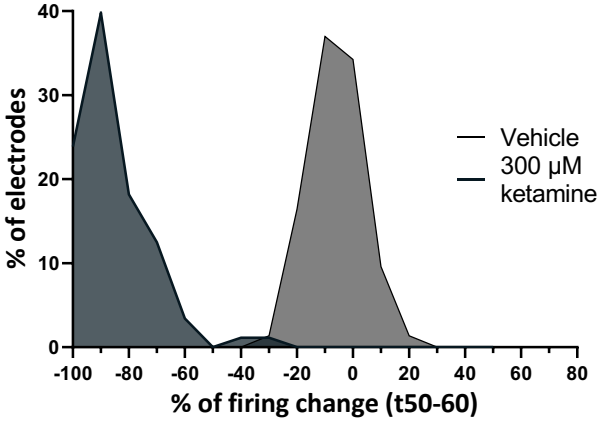












The spectra above figure the proportion of electrode displaying a change in the firing rate (in 10% increments) over 30-40 minutes after vehicle or ketamine application, when compared to the baseline period.

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VENTRAL TEGMENTAL AREA

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SUMMARY - Ventral tegmental area

Ventral tegmental area

- Information about the ventral tegmental area
- Materials & Methods

Results

- GABA receptors – Baclofen
- Dopaminergic receptors – Quinpirole
- Cyclooxygenase inhibitor - Acetaminophen
- Orexin receptors – Orexin-A, Suvorexant

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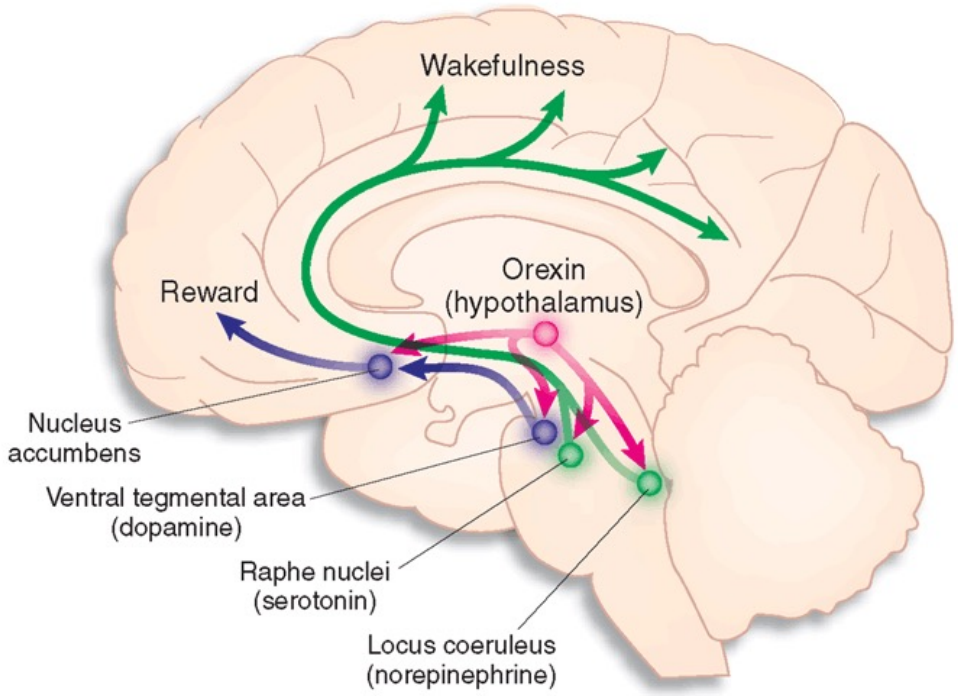
INTRODUCTION - Ventral tegmental area

Main summary
VTA summary

The ventral tegmental area (VTA) is the origin of the dopaminergic cell bodies and the source of dopamine pathways such as the mesocorticolimbic dopamine system. The VTA is implicated in the drug and natural reward circuitry, motivation, attention and memory (Chudasama & Robbins, 2004; Wise, 2004; Nicola et al. 2005) as well as several psychiatric disorders.

The ventral tegmental area (VTA) is a heterogeneous brain structure containing several neuronal populations, namely dopaminergic, gabaergic and some glutamatergic neurons.

The MEA technique does not allow to discriminate the nature of the recorded neurons (dopaminergic, gabaergic,…) from the action potentials waveform. However, $GABA_B$ receptor activation inhibits the firing of VTA dopaminergic neurons, but not VTA gabaergic neurons (Margolis et al, 2012). Baclofen - a selective agonist of $GABA_B$ receptors - is used to select electrodes recording dopaminergic neurons.



Nature

MATERIALS & METHODS - Ventral tegmental area

Area of recording – coronal rat VTA slices

Main summary
VTA summary

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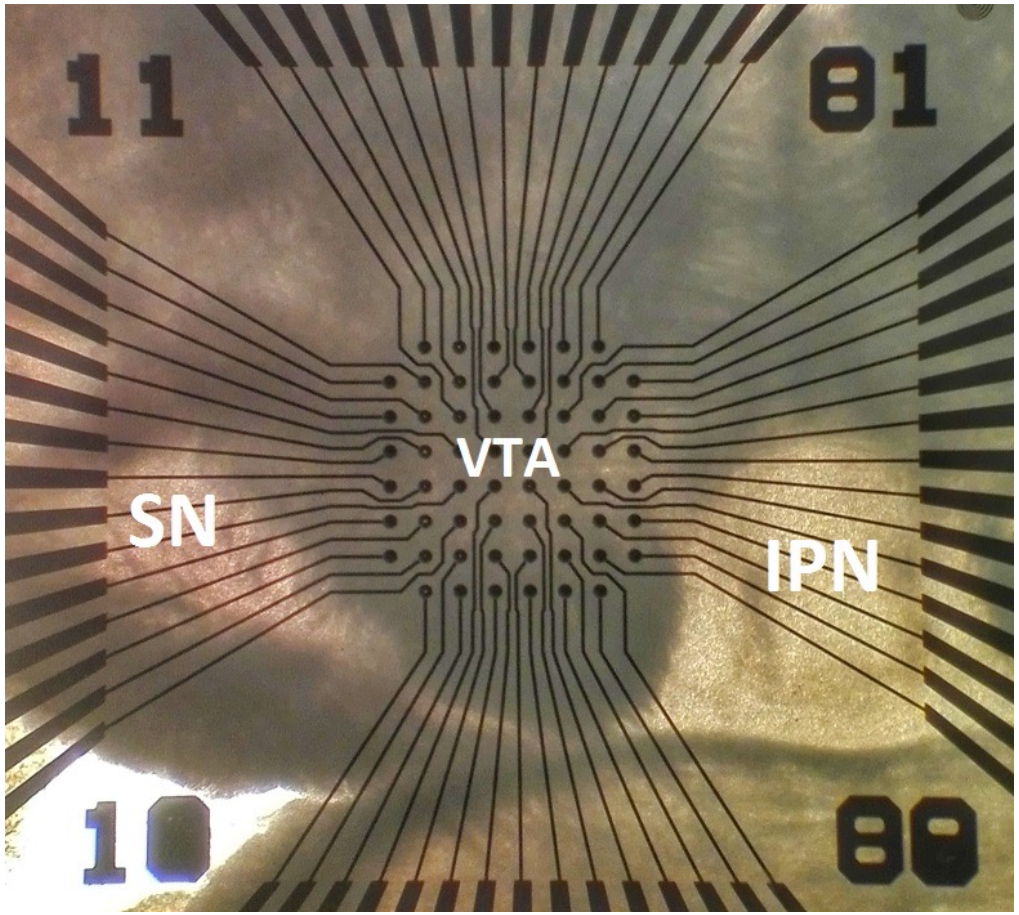
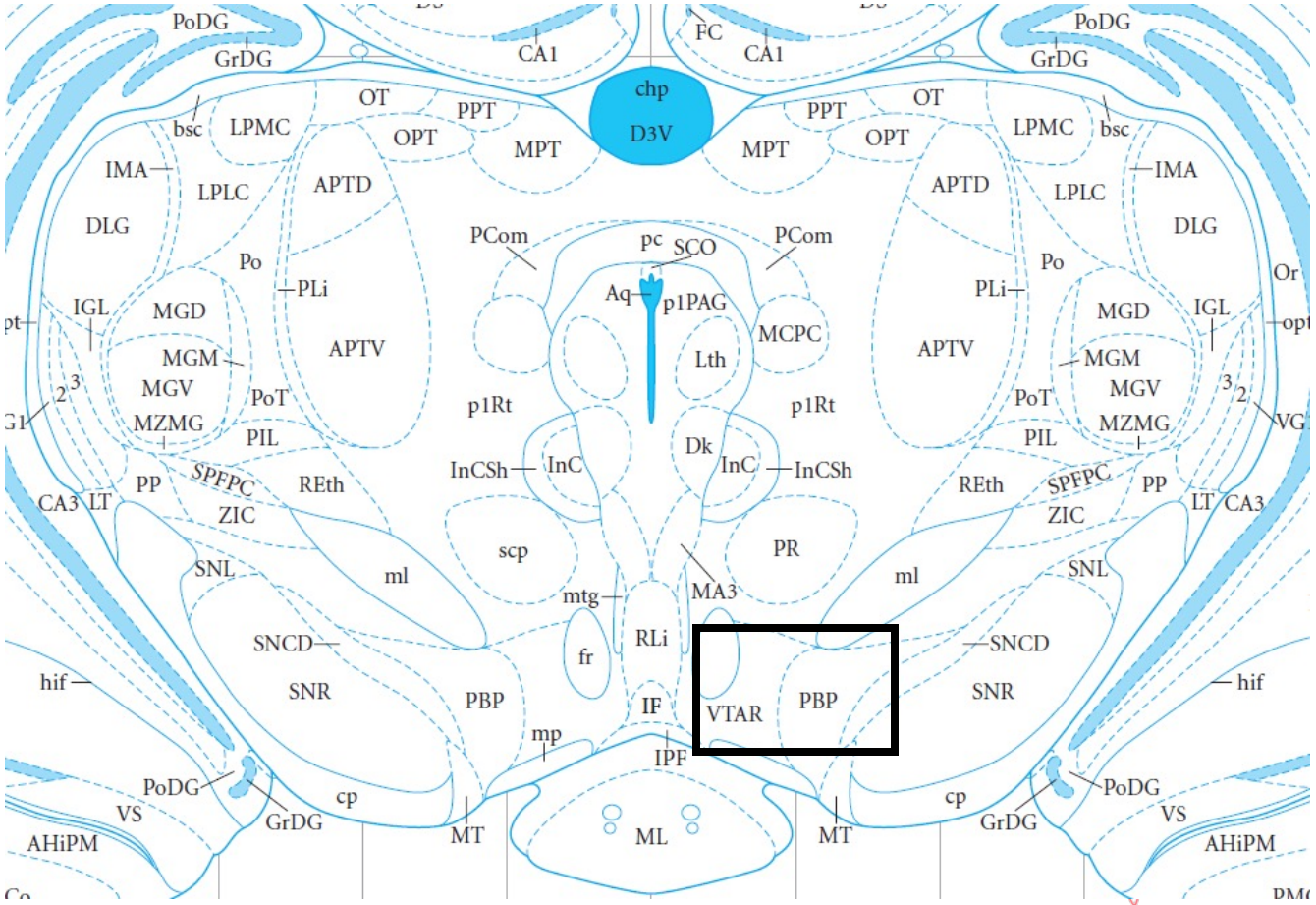




MATERIALS & METHODS - Ventral tegmental area

Analysis

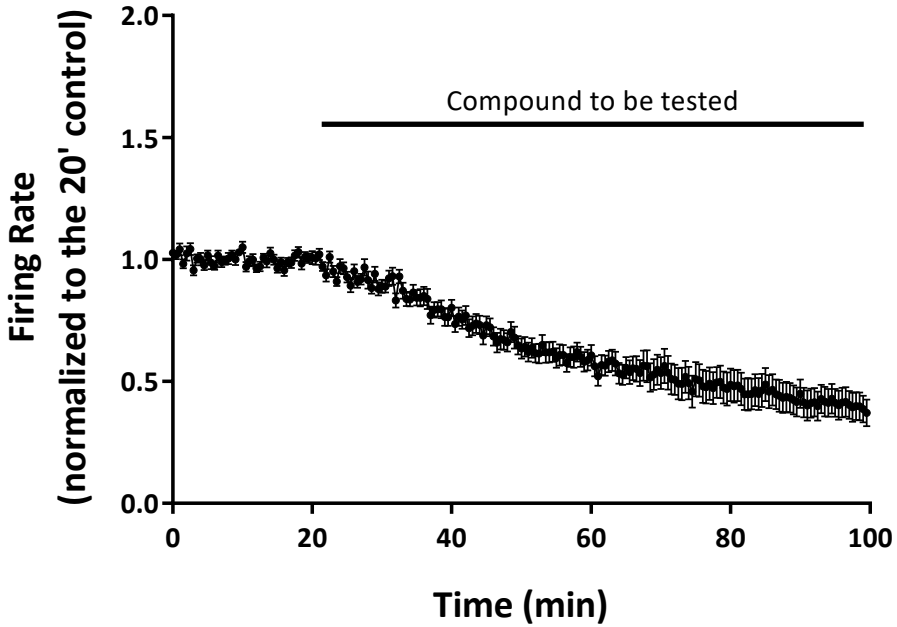
Main summary
VTA summary

Example of spontaneous firing

High-Pass filter
(set at 200 Hz)

Threshold detection
(-20 µV amplitude; dead time 2 ms)

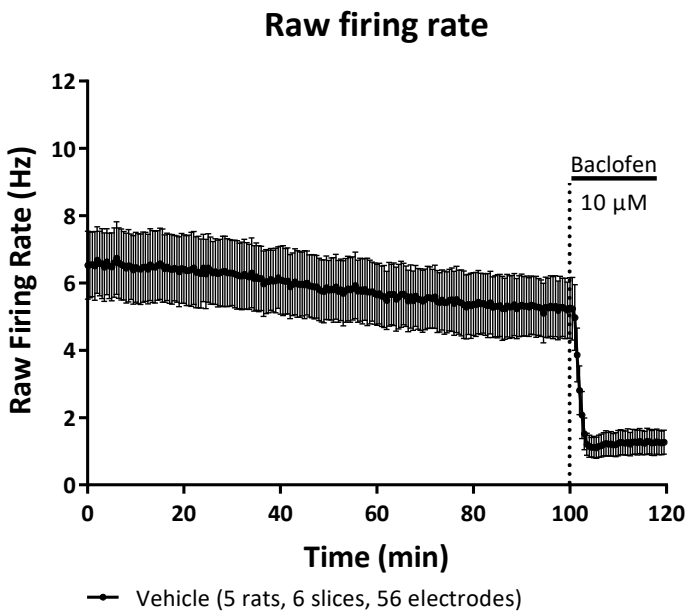
Firing rate
(% of firing change - averaged for 30 s bins)

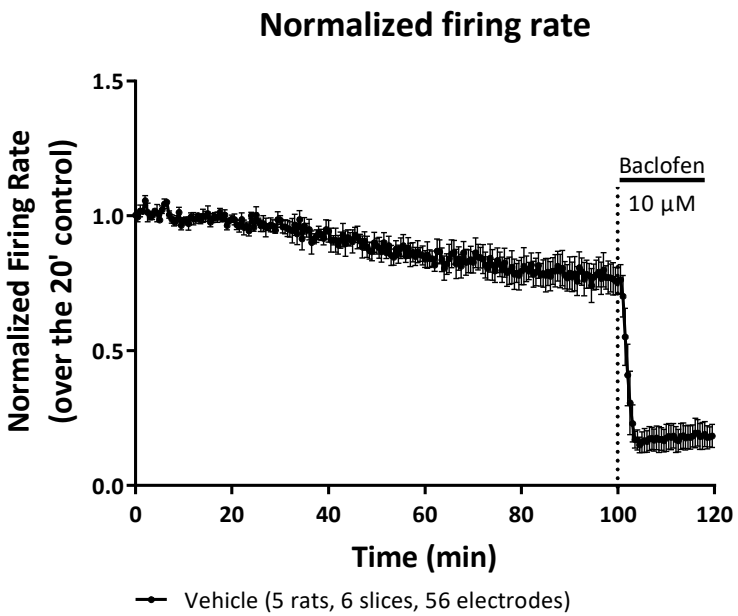


RESULTS - Ventral tegmental area

$GABA_B$ receptor

Main summary
VTA summary





- VTA neurons usually displayed a slight run down over 100 minutes of recording requiring vehicle slices recorded in parallel with compound-exposed slices.
- Baclofen – a selective agonist of $GABA_B$ receptors – is used to specifically inhibit an characterize the dopaminergic VTA neurons and a small proportion of glutamatergic neurons according to Margolis et al, 2012.

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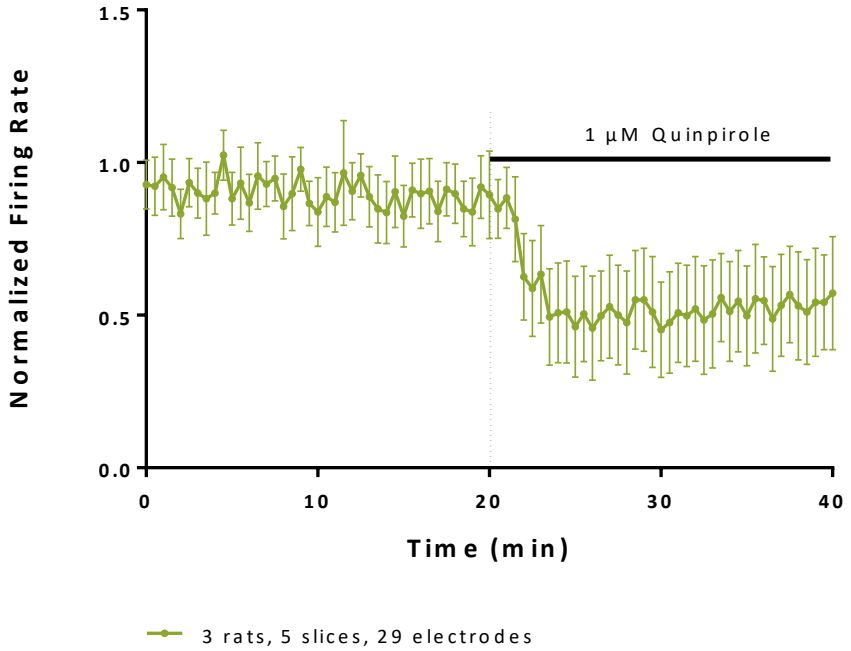
RESULTS - Ventral tegmental area

Dopaminergic D2 receptor

Main summary
VTA summary

Quinpirole



- 1 µM quinpirole - a selective D2 receptor agonist - decreased the spontaneous firing in the VTA by about 40 % after a 20-min application.

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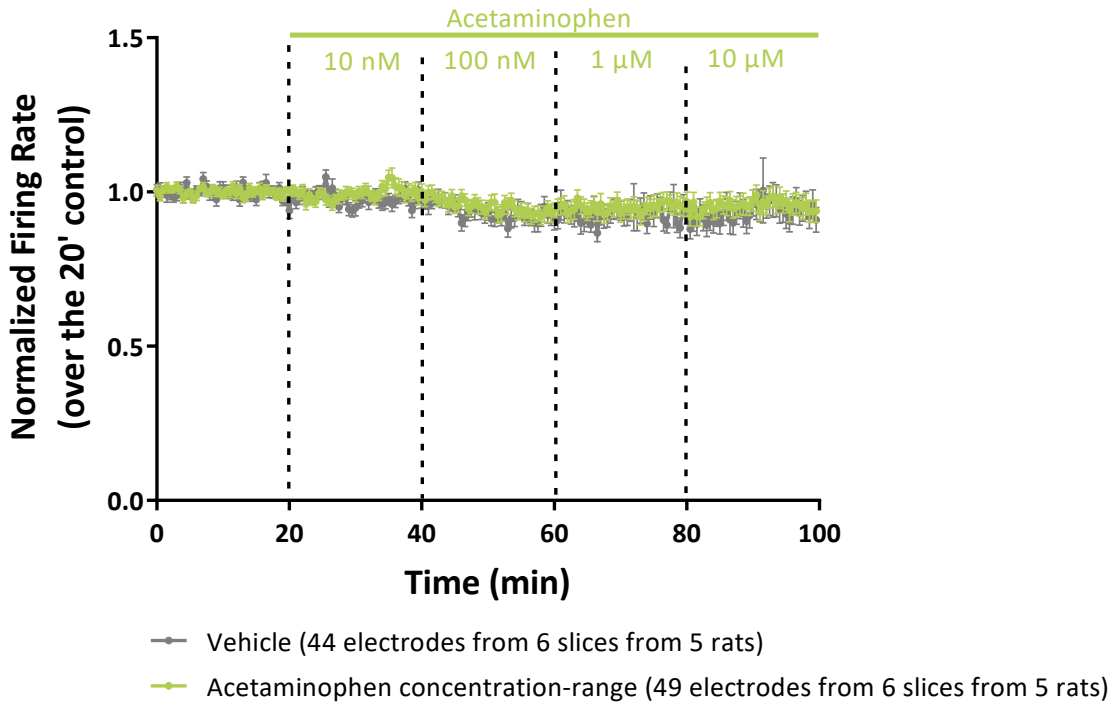
REFERENCE DATA - Ventral tegmental area

Cyclooxygenase

Main summary
VTA summary

Acetaminophen



- Acetaminophen, a cyclooxygenase inhibitor, did not modify the firing activity in the VTA

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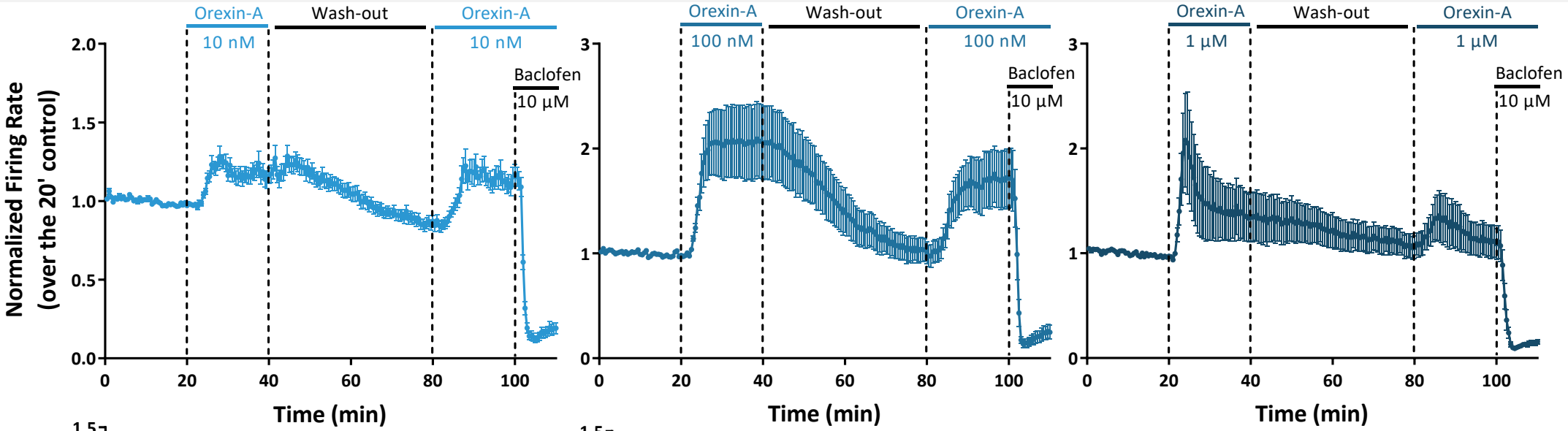
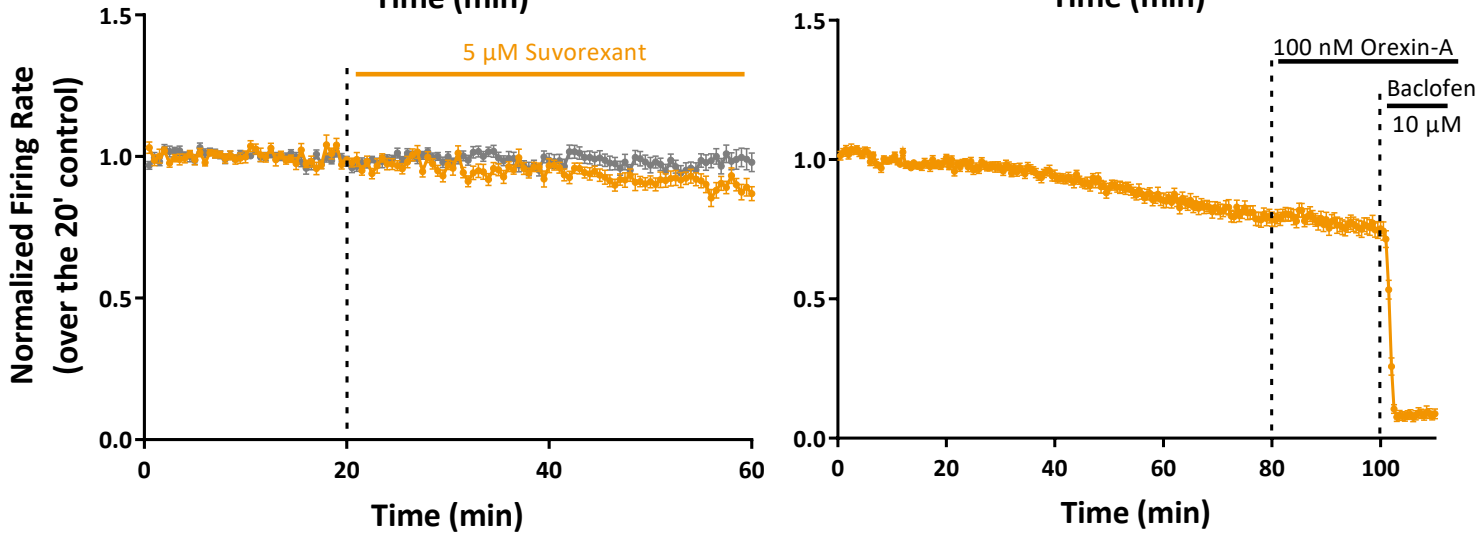
RESULTS - Ventral tegmental area

Main summary
VTA summary

Orexin receptors

Orexin-A
Suvorexant





- 10 nM Orexin-A (51 electrodes from 5 slices from 5 rats)
- 100 nM Orexin-A (40 electrodes from 5 slices from 5 rats)
- 1 µM Orexin-A (37 electrodes from 6 slices from 6 rats)

- Vehicle (36 electrodes from 6 slices from 6 rats)
- 5 µM Suvorexant (21 electrodes from 4 slices from 4 rats)
- In the presence of 5 µM suvorexant (40 electrodes from 6 slices from 5 rats)

- The VTA receives a massive input from the lateral hypothalamic area, including projections from neurons containing the neurotransmitter orexin-A (Fadel and Deutch, 2002). Orexin-A significantly and concentration-dependently increased the firing activity in the VTA.
- Suvorexant – a dual orexin receptor antagonist – did not modify the spontaneous firing of VTA neurons but blocked the action of orexin.

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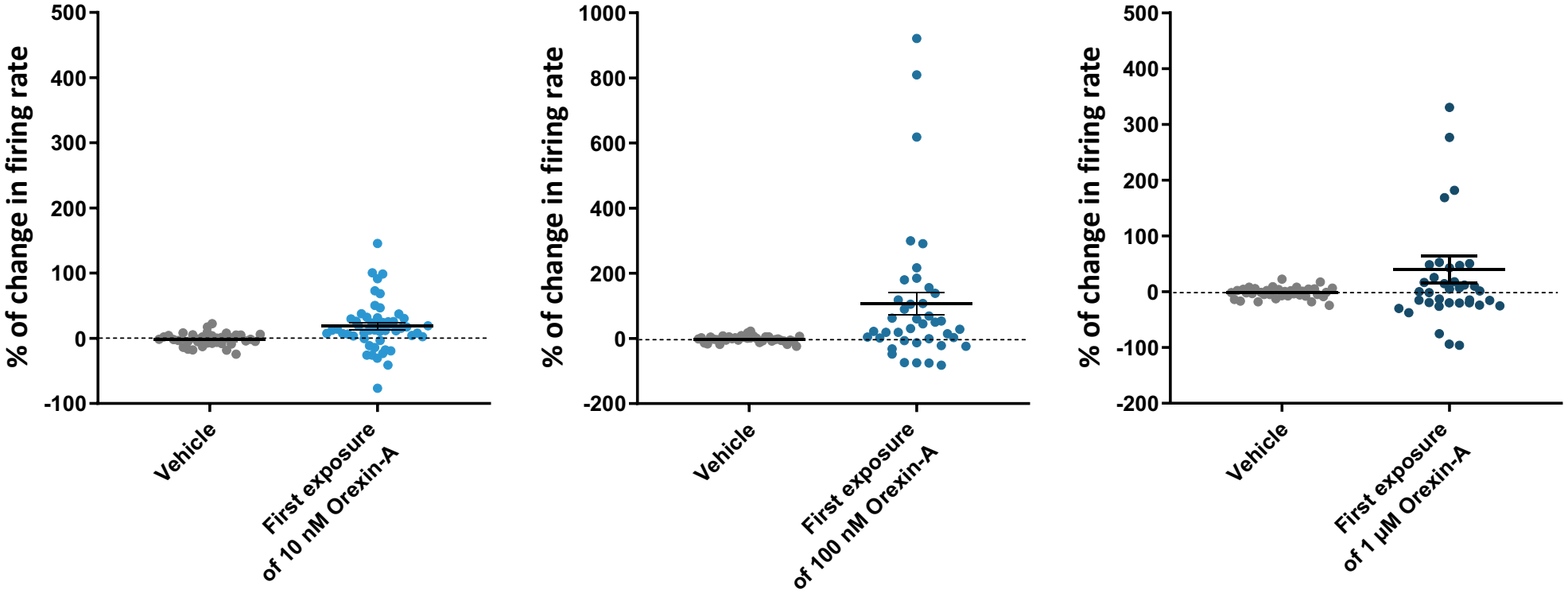
RESULTS - Ventral tegmental area

Main summary
VTA summary

Orexin receptors

Orexin-A



- Scatter plot comparing the % of change in firing rate after vehicle or orexin-A application, for each individual electrode.

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SUBSTANTIA NIGRA

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SUMMARY - Substantia Nigra

Main summary

Substantia Nigra

- Information about the substantia nigra
- Materials & Methods

Results

- Dopaminergic receptors – Dopamine / Quinpirole
- L-type calcium channel – Isradipine

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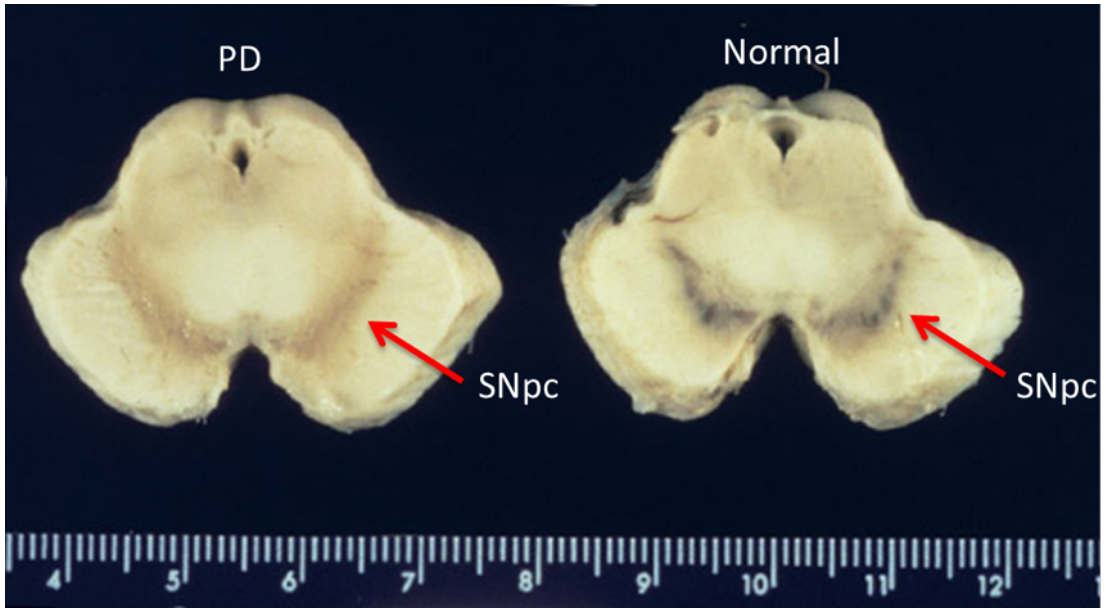
INTRODUCTION - Substantia Nigra

Main summary
SN summary

MEA recordings in midbrain slices capture the high degree of complexity in the firing of SN neurons and offer a new option in the investigation of the dopaminergic systems in vitro. Multiple neurons can be recorded during a single experiment, enabling the investigation of new targets for the pharmacological treatment of dopamine-dependent neurological disorders, such as Parkinson's disease and other movement disorders.

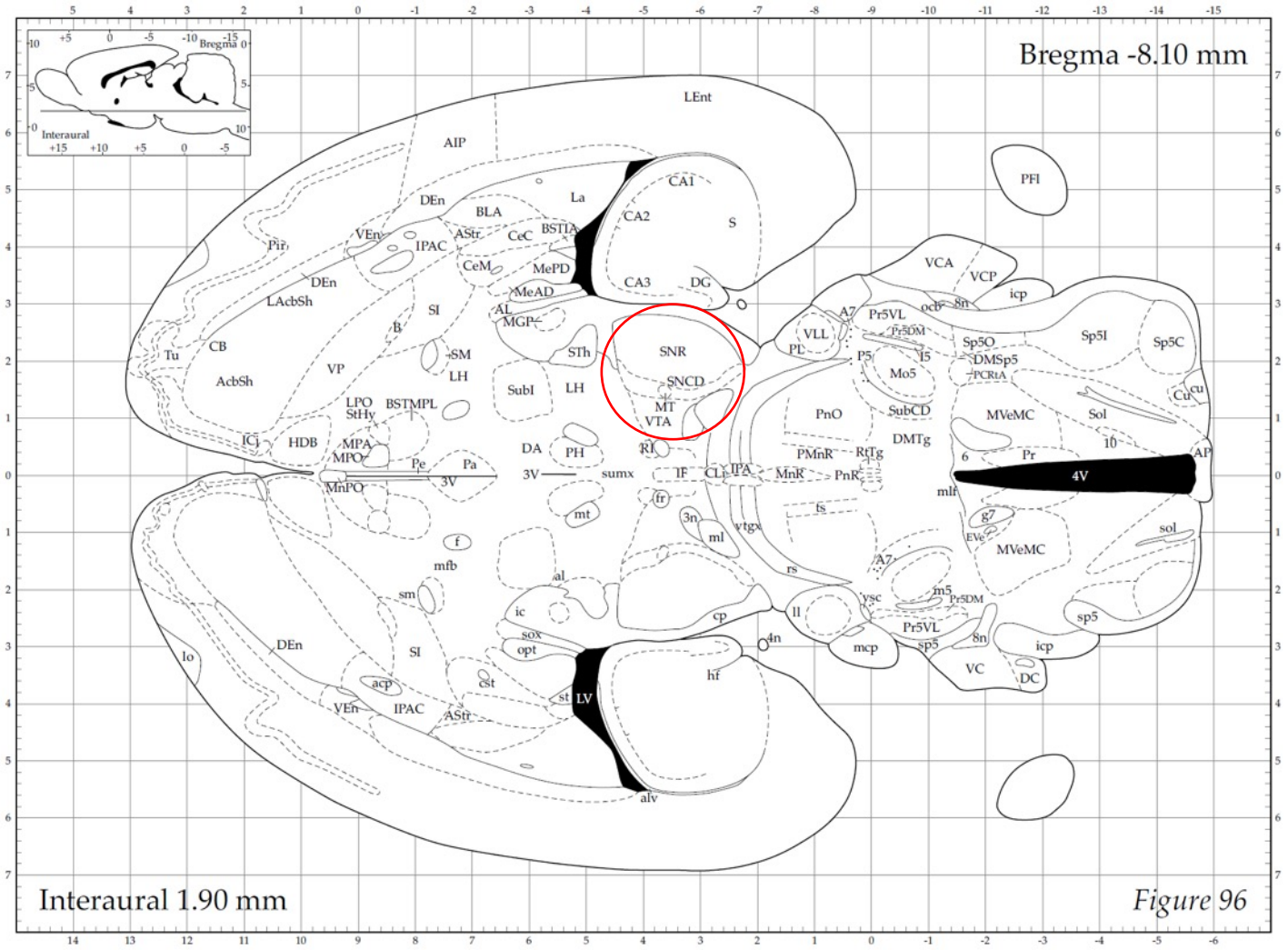


Loss of dopaminergic neuron in substantia nigra pars compacta (SNpc)

MATERIALS & METHODS - Substantia Nigra

Main summary
SN summary

Area of recording – Horizontal rat SN slices



The substantia nigra (SN) nucleus can be clearly recognized within horizontal/parasagittal brain slices.

The firing activity of substantia nigra pars compacta (SNc) and reticulata (SNr) neurons can be successfully recorded and analyzed thanks to the MEA technique.

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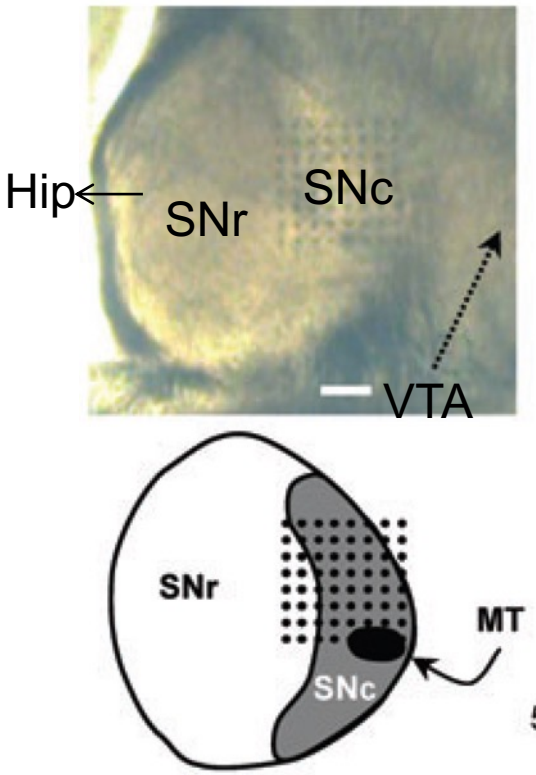
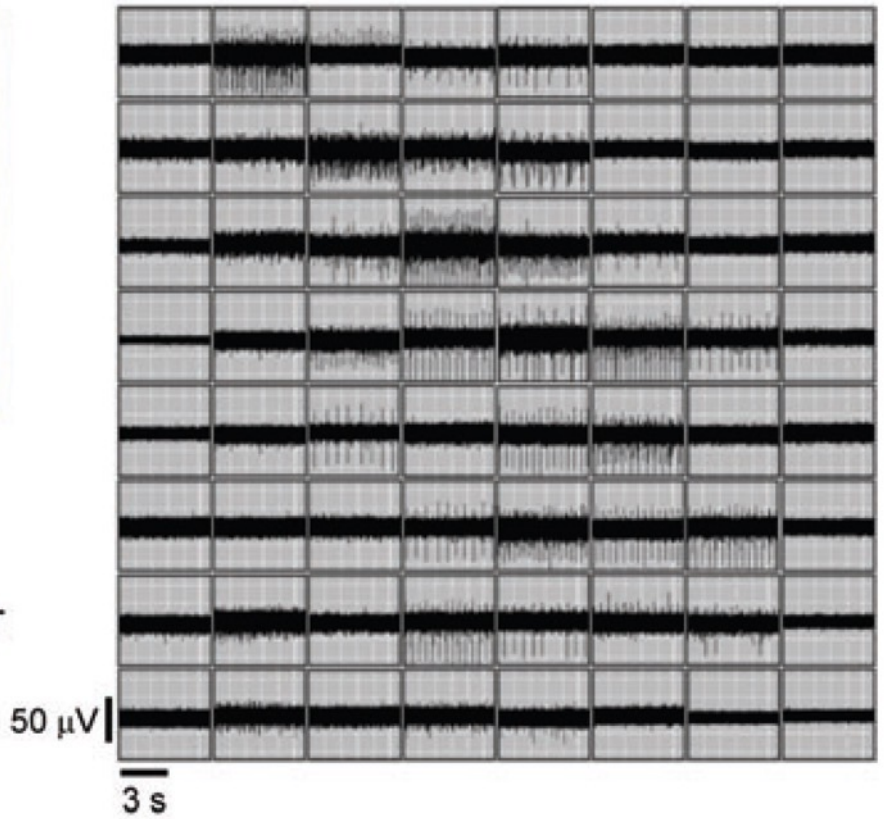
MATERIALS & METHODS - Substantia Nigra

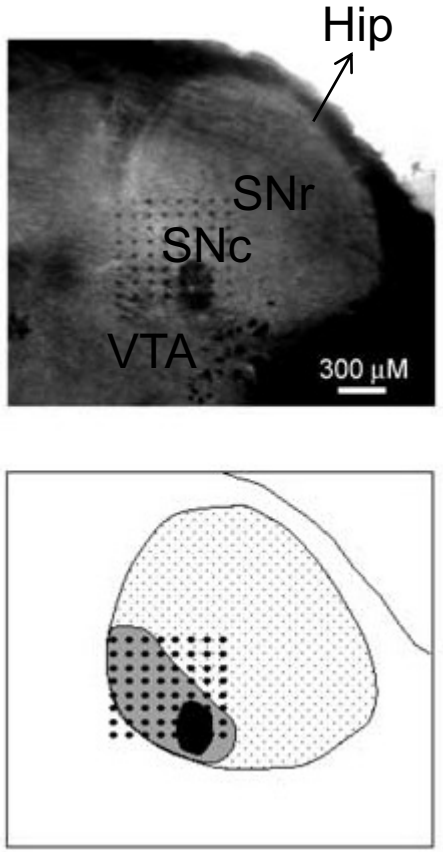
Main summary
SN summary

Literature data

Clarify which figure of which article







Photograph and schematic drawing of a midbrain slice placed over an array of planar multi-electrodes. SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; MT: medial terminal nucleus of the accessory optic tract. Figure adapted from Berreta et al., 2010.

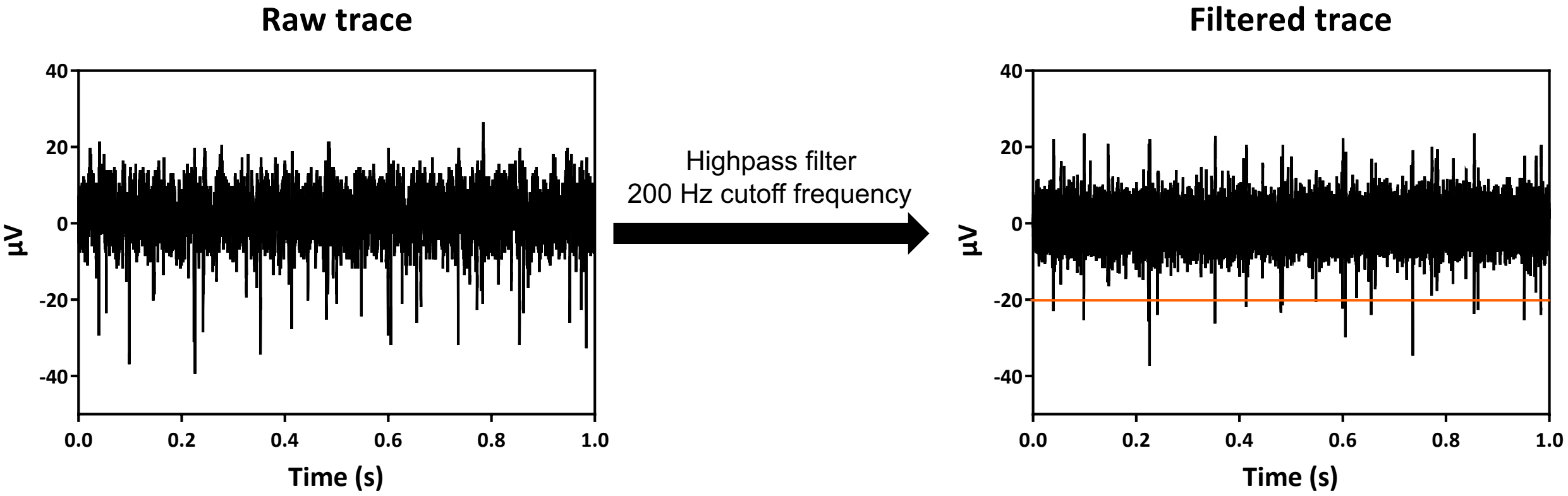
300 µm thick horizontal slices placed on a 8x8 MEA (100 µm distant electrodes)

Berreta et al., 2010
Geracitano et al.,2005

MATERIALS & METHODS - Substantia Nigra

Analysis

Main summary
SN summary



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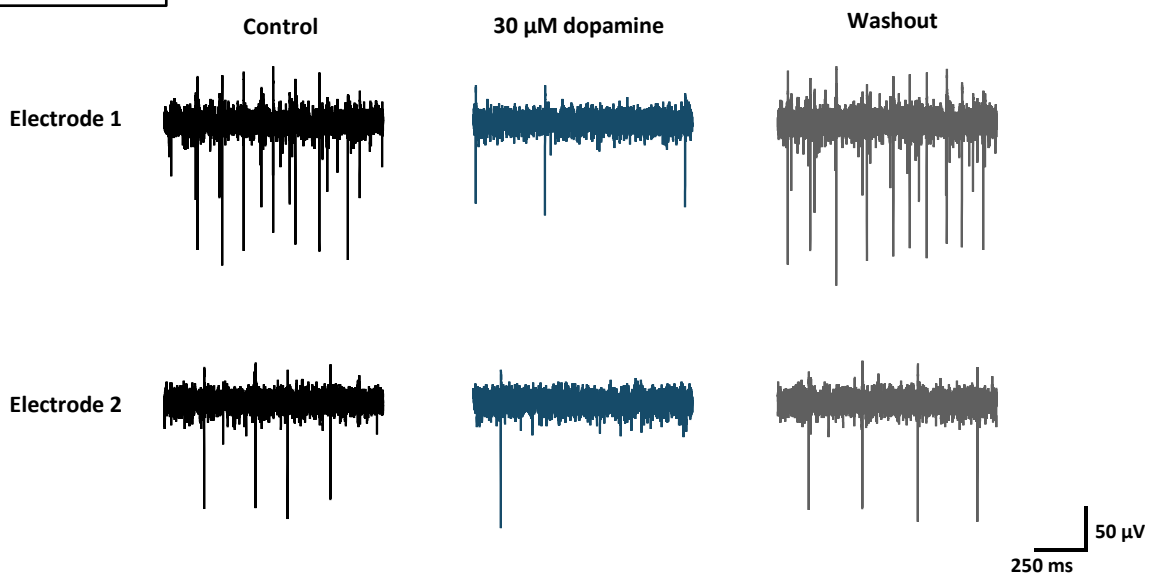
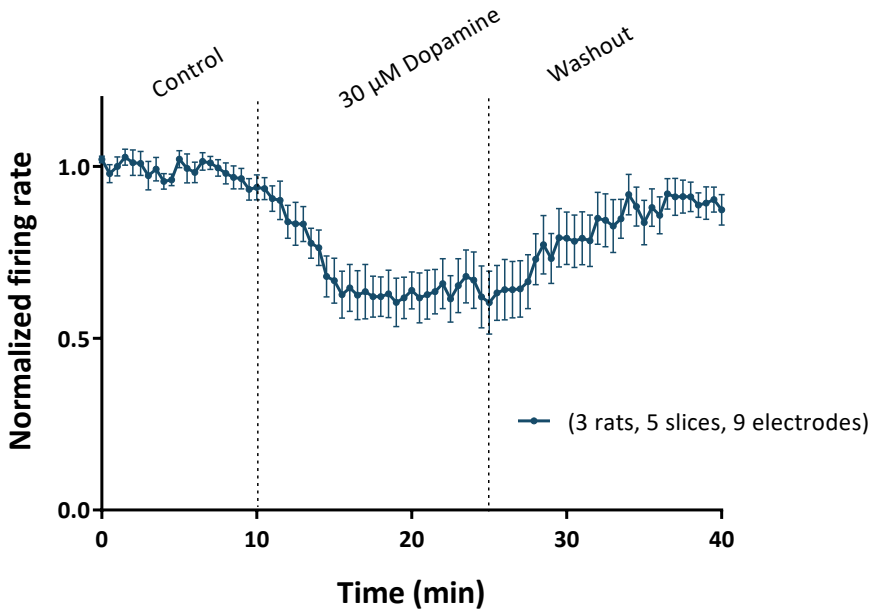
RESULTS - Substantia Nigra

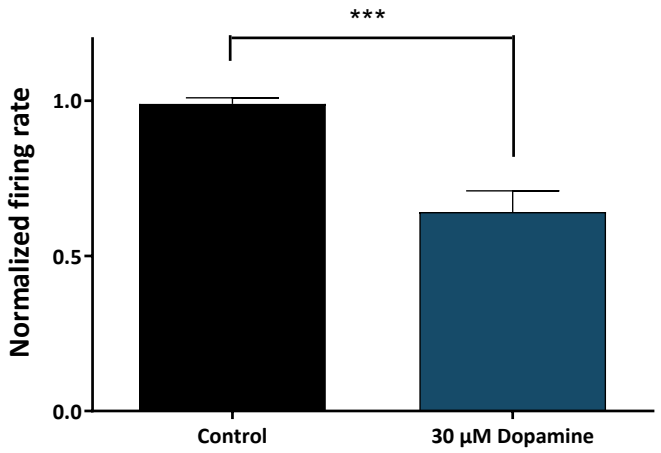
Main summary
SN summary

Dopaminergic receptors

Dopamine







Representative traces showing the effect of Dopamine on SNc neurons spontaneous firing.

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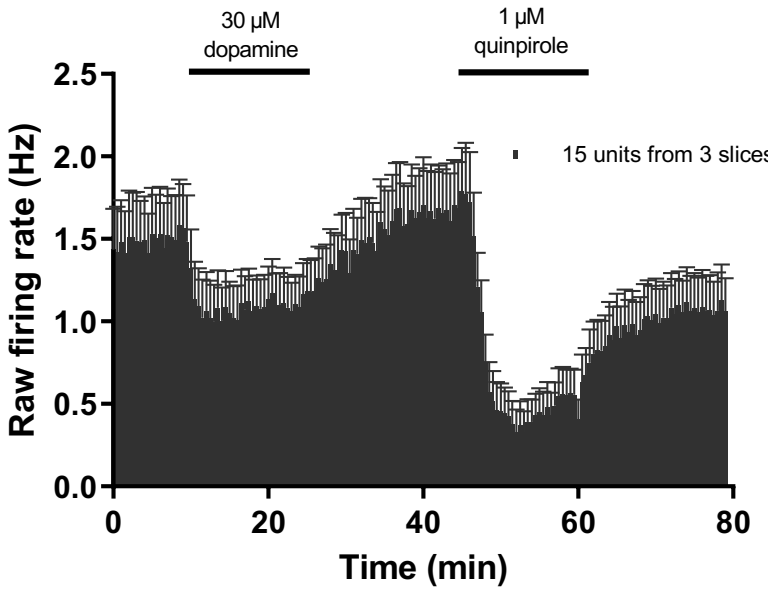
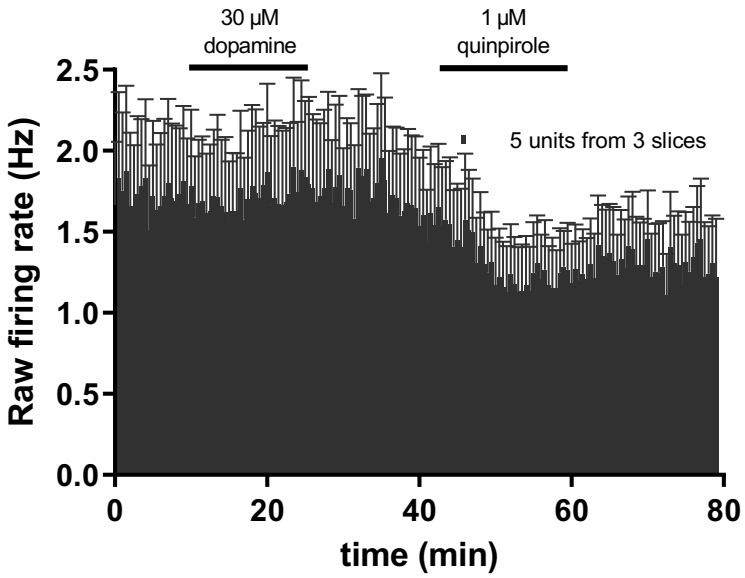
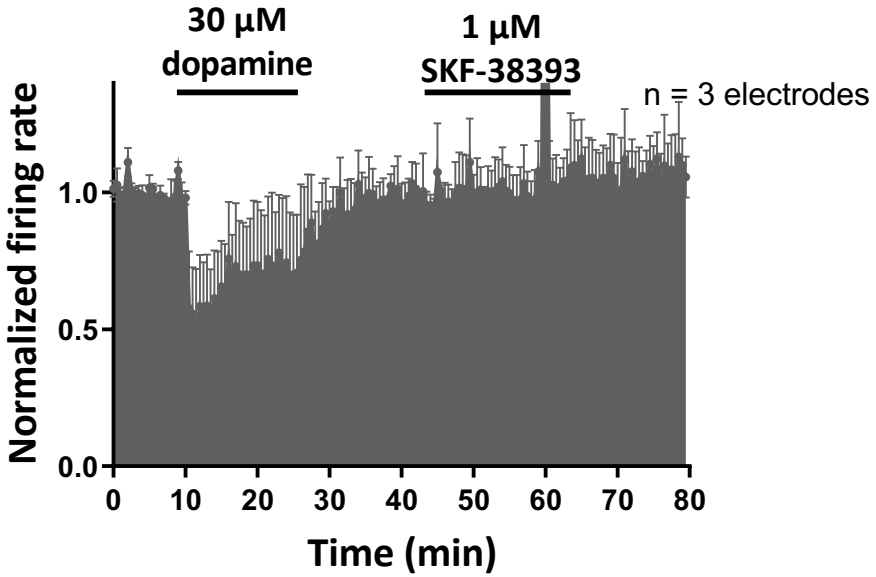
RESULTS - Substantia Nigra

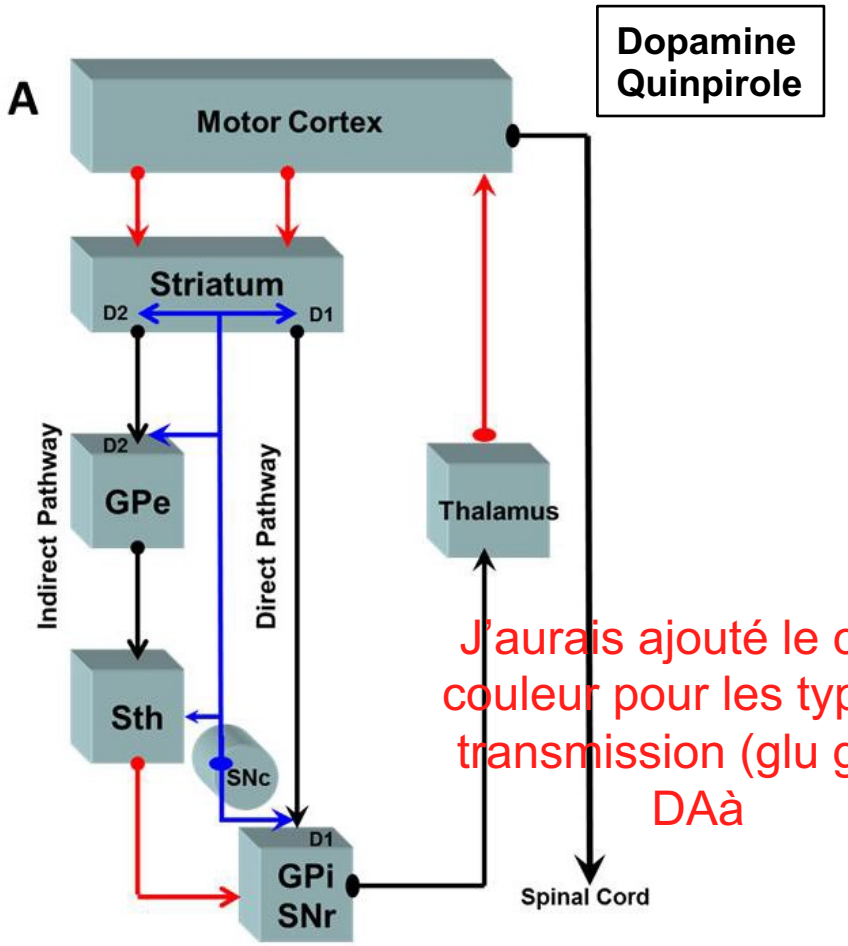
Dopaminergic receptors







Quinpirole (a D2R agonist) particularly inhibits firing activity of dopamine-sensitive neurons and SKF-38393 - Selective D1-like agonist- does not modulate the firing activity in the SNc



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Basal ganglia nuclei direct and indirect pathways

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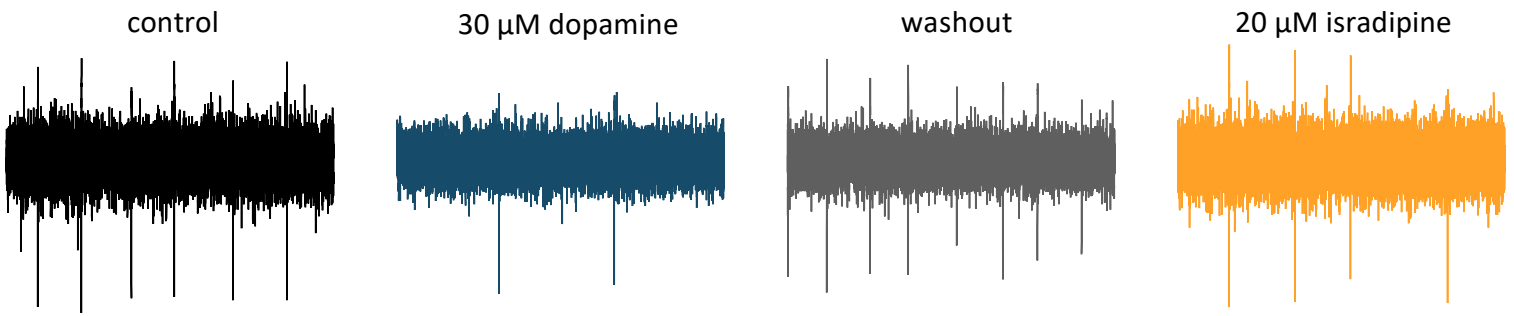
RESULTS - Substantia Nigra

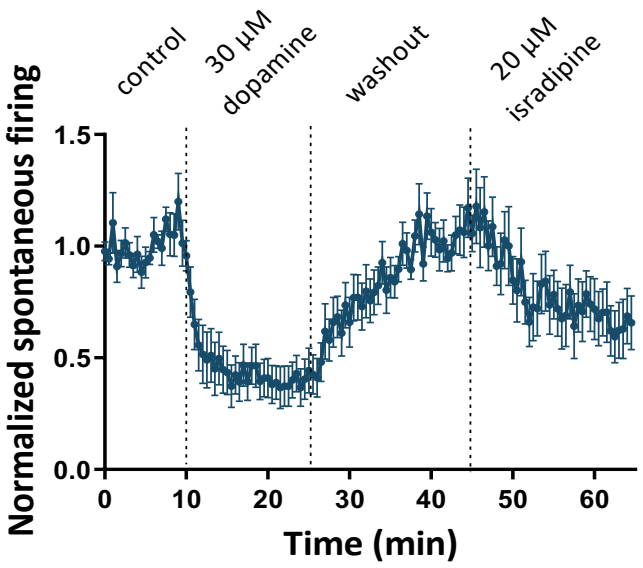
L-type calcium channel

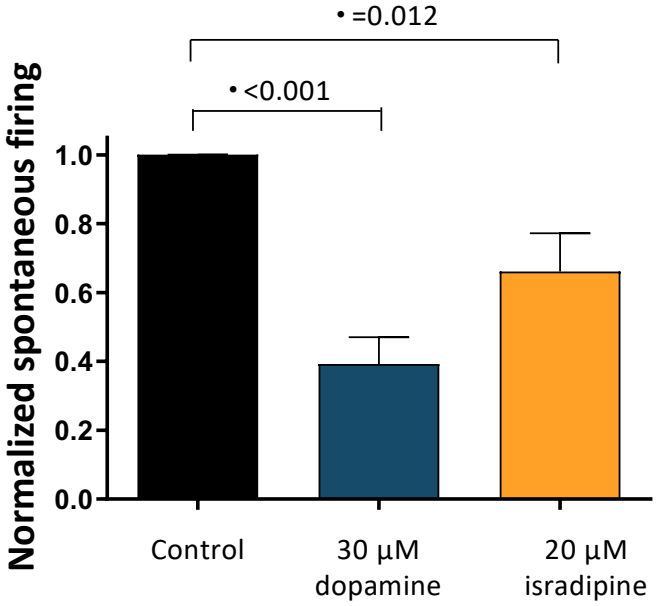
Dopamine, isradipine

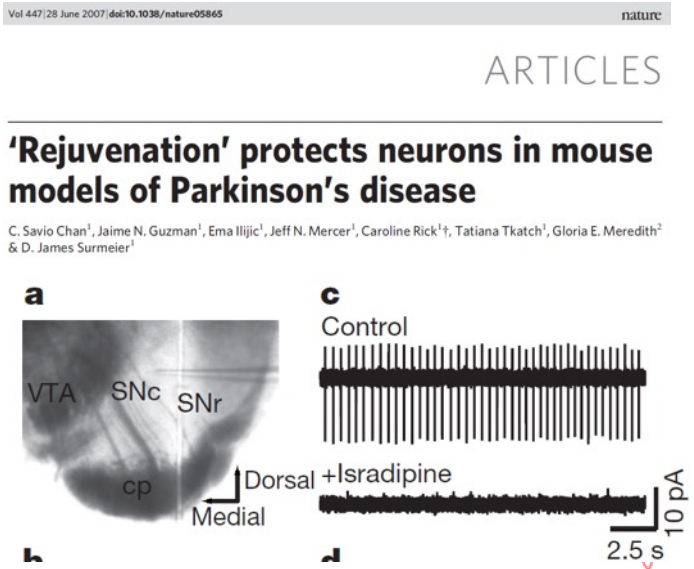


Representative traces showing the effect of Dopamine and Isradipine on SNc neurons spontaneous firing.

Neurons sensitive to dopamine are responsive to isradipine (L-type calcium channel blocker)







30 µM dopamine (15min) + 20 µM isradipine (20min)
(5 rats, 6 slices, 11 eletrodes)

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SUBTHALAMIC NUCLEUS

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SUMMARY - Subthalamic nucleus

Main summary

Subthalamic nucleus

- Information about the subthalamic nucleus
- Materials & Methods

Results

- Ionotropic glutamate receptors – NMDA
- Metabotropic glutamate receptors – ACPD
- Dopaminergic receptors – Quinpirole

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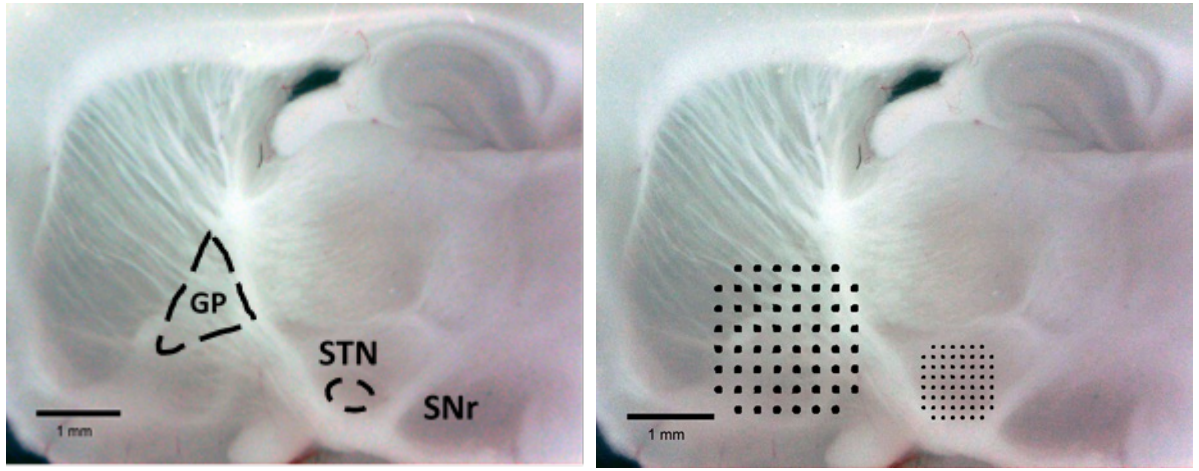
INTRODUCTION - STN

Main summary
STN summary

The basal ganglia (BG) nuclei are a set of interconnected subcortical brain nuclei primarily involved in movements and motivational aspects of motor behavior. The indirect pathway successively involves the globus palidus (GP), the subthalamic nucleus (STN) and the substantia nigra pars reticulata (SNr).

The STN firing activity can be recorded *in vitro*, from acute brain slices (see Neuroservice preliminary data below). Literature has shown that firing activity in the STN can be enhanced by activation of NMDA or metabotropic glutamate receptors (Beurrier, 1999; K.C Loucif, 2005). Moreover, Dopamine and Quinpirole injection in the GP reduced the firing rate of majority of STN and SNr neurons (Omar Mamad, 2015).



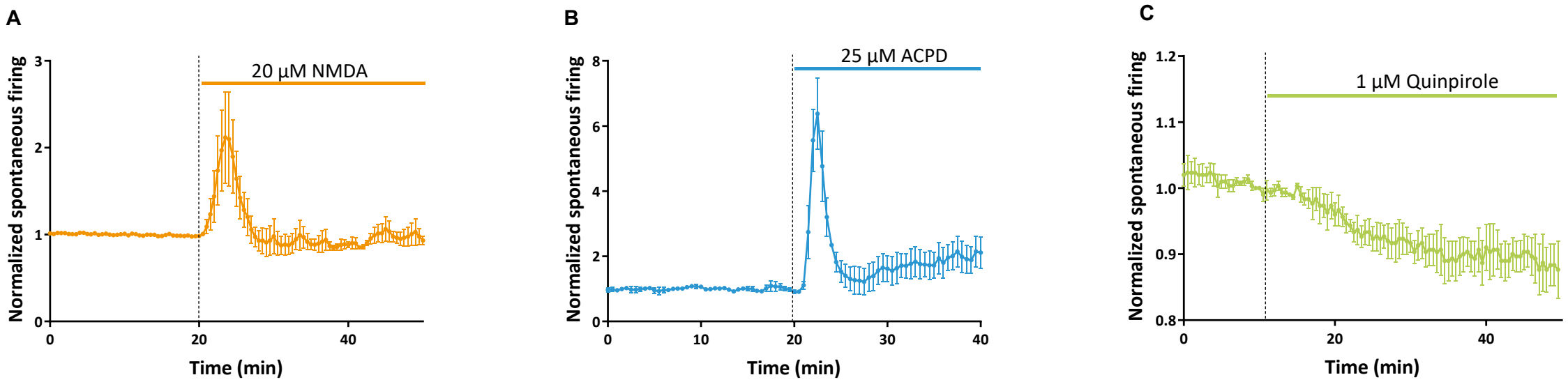
Left picture illustrates the position of basal ganglia nuclei within a parasagittal rat brain slice. On the right picture is shown the area covered by electrodes for a 200 3D MEA (electrodes spaced by 200 µm, centred on the GP), or for a 100 3D MEA (electrodes spaced by 100 µm, centred on the STN, Neuroservice pictures).

RESULTS - Subthalamic nucleus

Dopaminergic receptors, ionotropic & metabotropic glutamate receptors

Main summary
STN summary

NMDA, ACPD, quinpirole



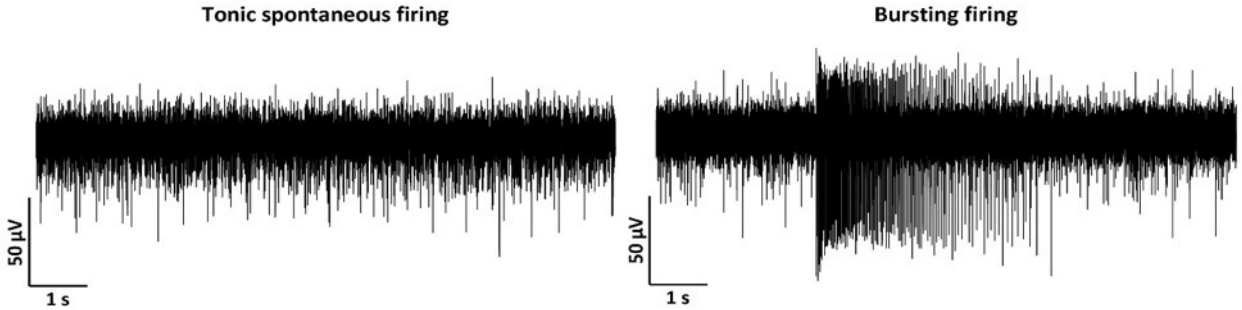


Illustration of the different firing patterns observed in the STN (in 3.5 mM K^+ aCSF, Neuroservice data).

(A) and (B) Exposure to 20 µM NMDA (NMDA glutamate receptors agonist) and 25 µM ACPD (a group I and II metabotropic receptor agonist) strongly increased the firing activity but their effect rapidly desensitized. (C) Exposure to 1 µM Quinpirole (D2 dopaminergic receptor agonist) decreased the firing rate in the STN.

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PERIAQUEDUCTAL GREY MATTER

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SUMMARY - Periaqueductal grey matter

Main summary

Periaqueductal grey matter

- Information about the periaqueductal grey matter
- Materials & Methods

Results

- $GABA_A$ receptors antagonists – Bicuculline, CGP-55845
- Opioid receptors – DAMGO, Fentanyl, Morphine, Oxycodone
- Cyclooxygenase – Acetaminophen

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INTRODUCTION - Periaqueductal grey matter

Main summary
PAG summary

The periaqueductal grey matter (PAG) is involved in the modulation of pain and analgesia (Finn et al., 2003).

The ventrolateral periaqueductal gray (vPAG) is crucial for the development of antinociceptive tolerance to morphine. Microinjection of morphine or DAMGO into the vPAG produces antinociception and repeated intra-vPAG administration of morphine produces tolerance. (Lane et al., 2005; Morgan et al., 2006a; Tortorici et al., 1999).

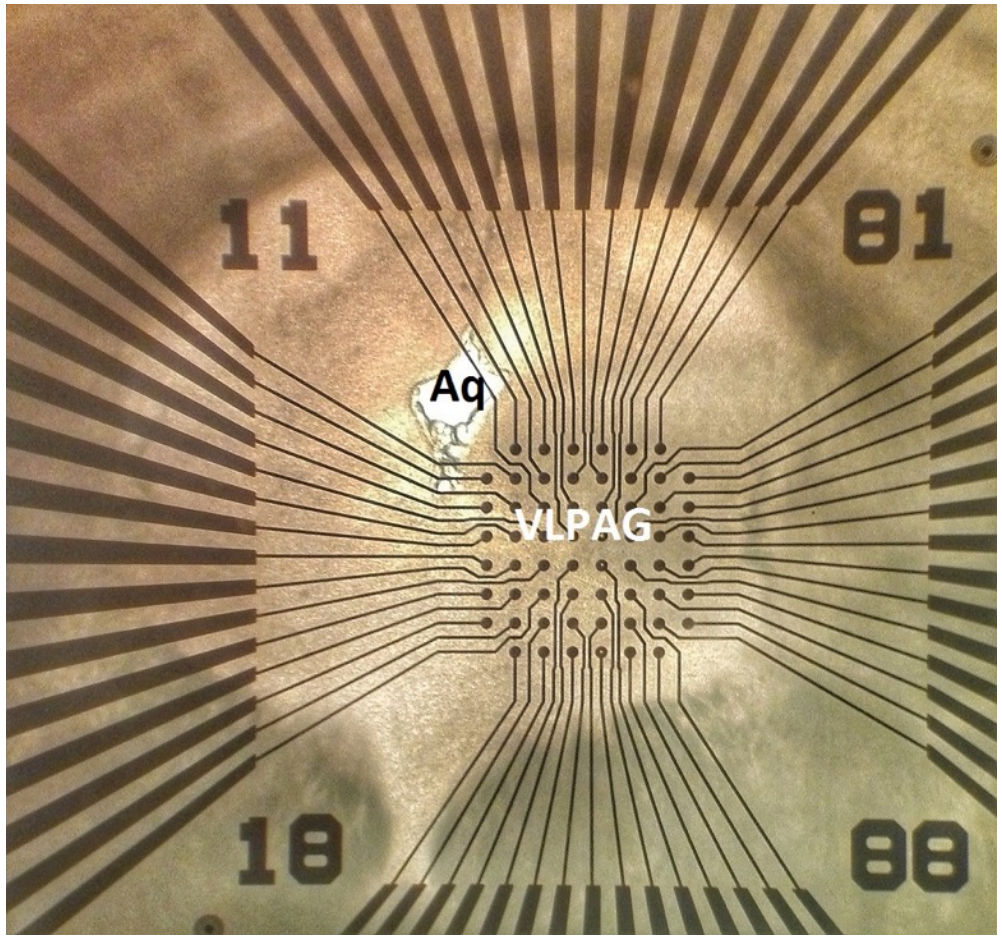
It is also a major site of analgesic action by exogenous cannabinoid agonists. The physiological significance of endocannabinoids in the PAG was previously highlighted in a study by Hohmann *et al*. (2005), who showed that the non-opioid component of stress-induced analgesia is mediated by endocannabinoids.

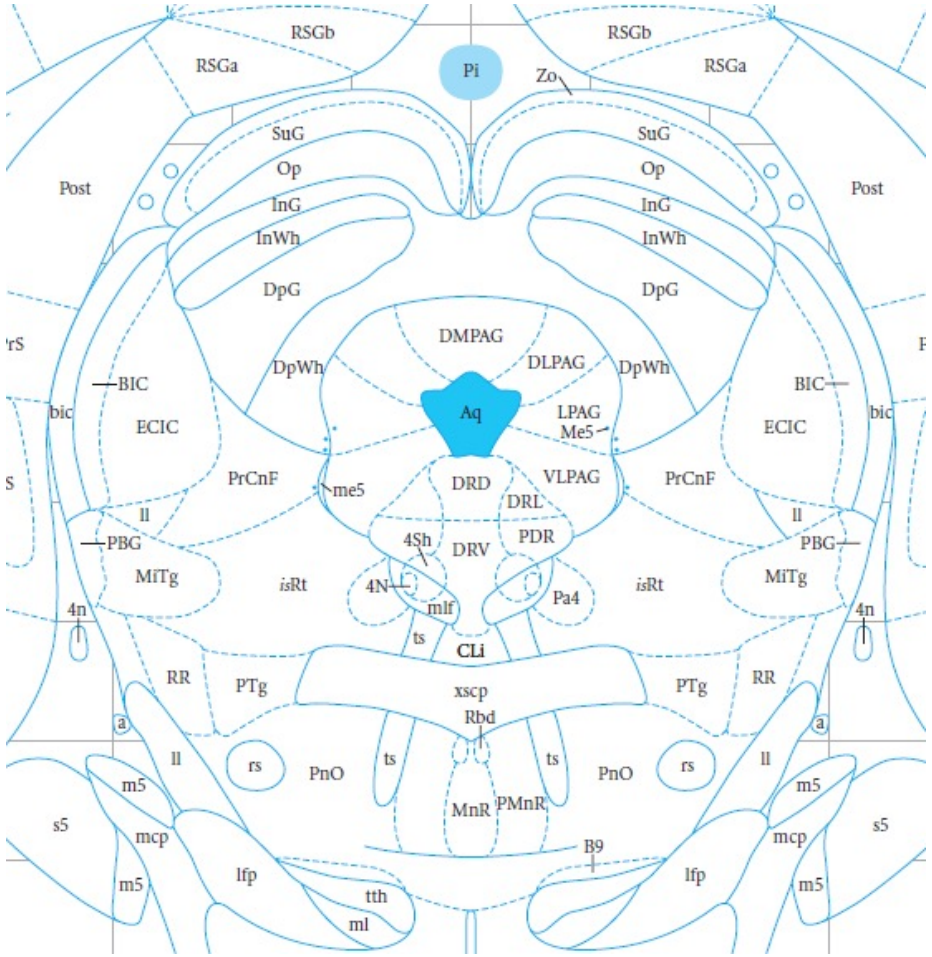
The periaqueductal grey matter (PAG) is of interest in pain and drug tolerance purpose. Moreover in that purpose, different mechanisms are engaged between spinal cord and periaqueductal grey matter.

MATERIALS & METHODS - Periaqueductal grey matter

Area of recording – coronal rat PAG slices

Main summary
PAG summary





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MATERIALS & METHODS - Periaqueductal grey matter

Analysis

Main summary
PAG summary

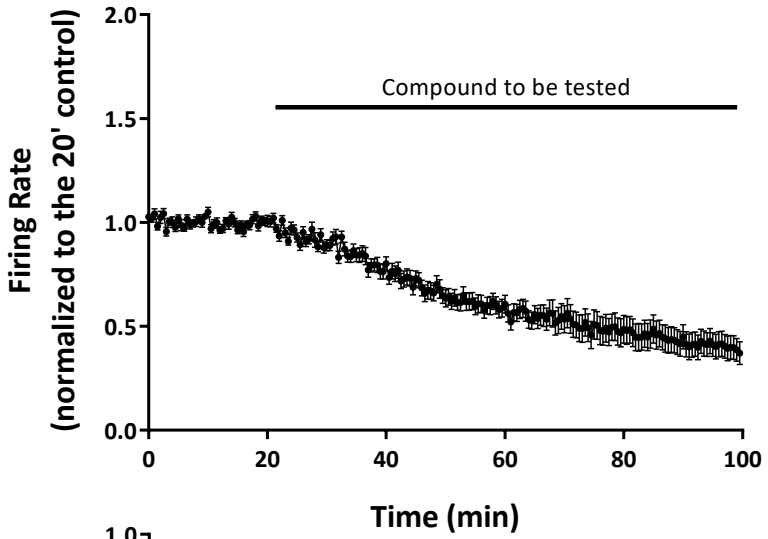
Example of spontaneous firing

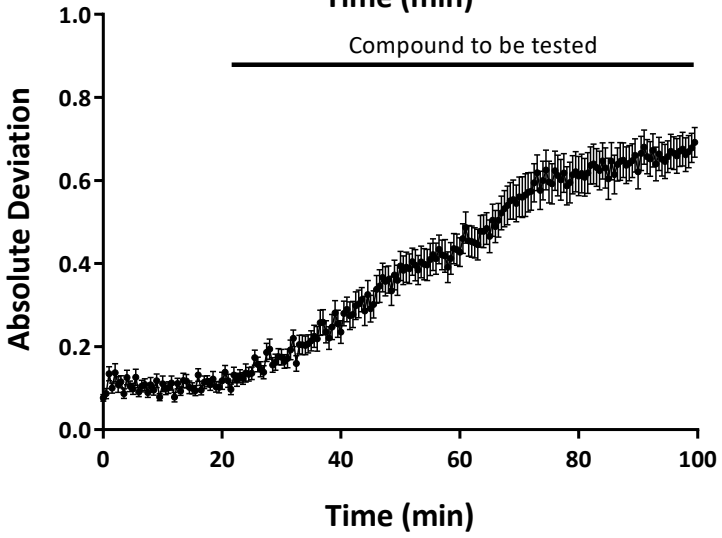
High-Pass filter
(set at 200 Hz)

Threshold detection
(-20 µV amplitude; dead time 2 ms)

Firing rate
(% of firing change - average for 30 s bins)

Absolute deviation*
(absolute value for positive and negative modulations are taken into considerate)





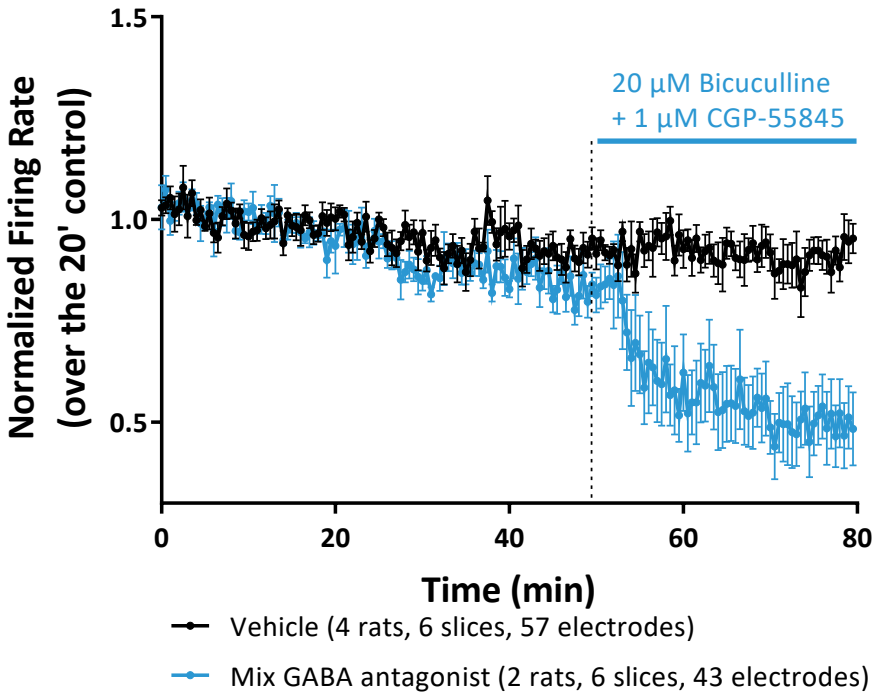
*** region containing different types of neurons, opposite effect can be observed according to the electrode. Measurement of absolute deviation is useful to compare the effect of a compound to the one of vehicle.**

RESULTS - Periaqueductal grey matter

$GABA_{A\ \&\ B}$ receptors

Main summary
PAG summary

Bicuculline
CGP-55845



- In vehicle slices (black circle), the spontaneous firing rate was recorded over a 80-minute period and remained quite stable. At the end of experiment the normalized firing rate was 0.95 ± 0.04.

- When applied alone (blue circle) the mix of $GABA_A$ and $GABA_B$ antagonists rapidly decreased the spontaneous firing rate to reach 0.48 ± 0.09 after a 30 minute period exposure, corresponding to a decrease of 42 %.

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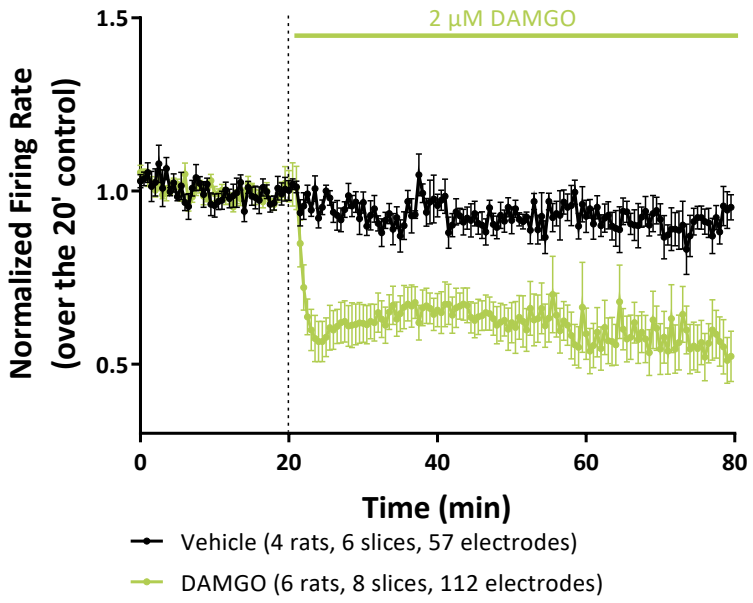
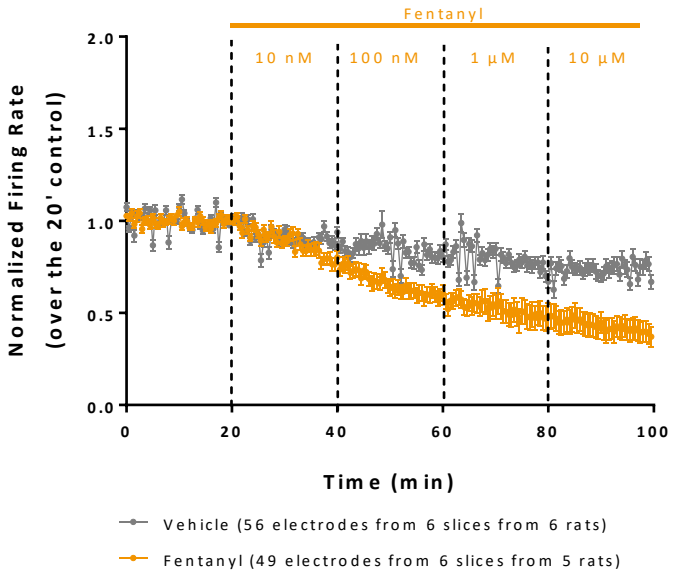
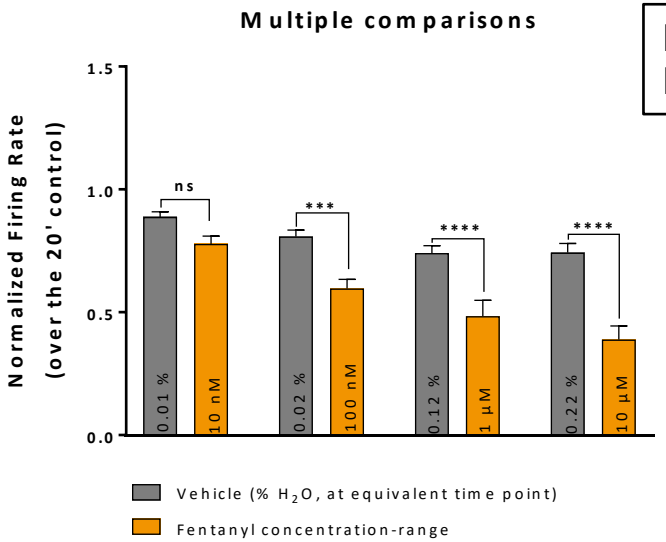
RESULTS - Periaqueductal grey matter

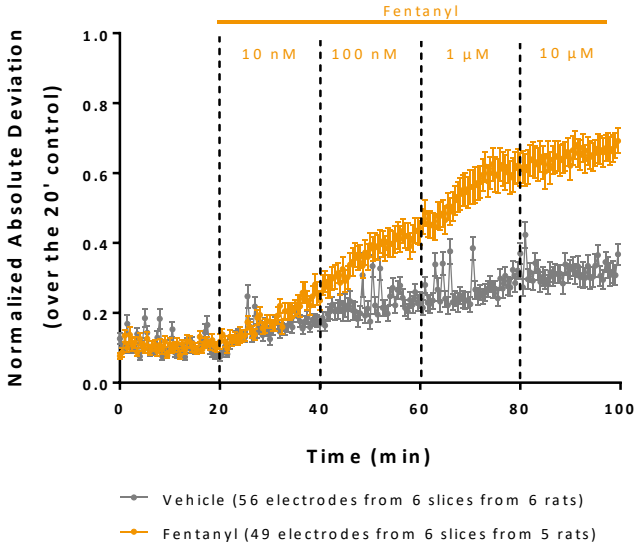
Opioids receptors

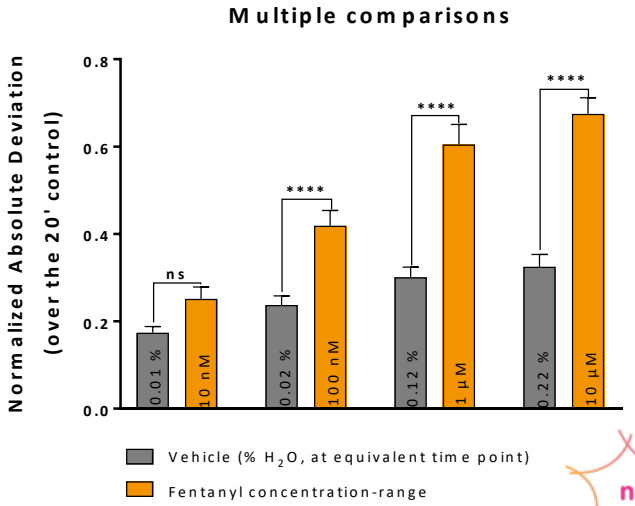
Main summary
PAG summary











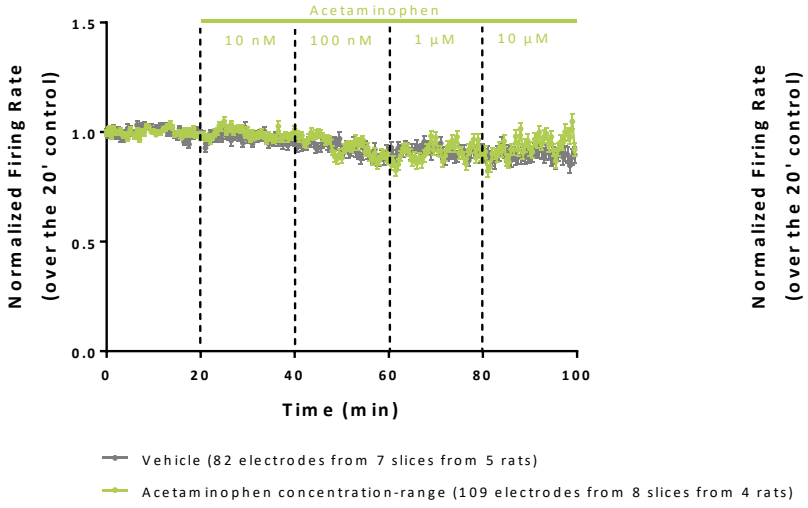
- DAMGO – a µ-opioid receptor agonist – substantially decrease the firing rate in VlPAG.
- Fentanyl – a µ-opioid receptor agonist – decrease on average the firing rate in VlPAG. But some electrodes displayed opposite effect.

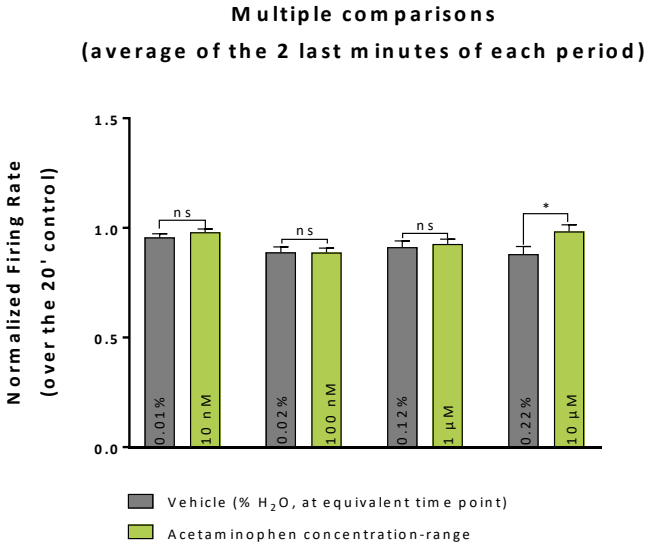
RESULTS - Periaqueductal grey matter

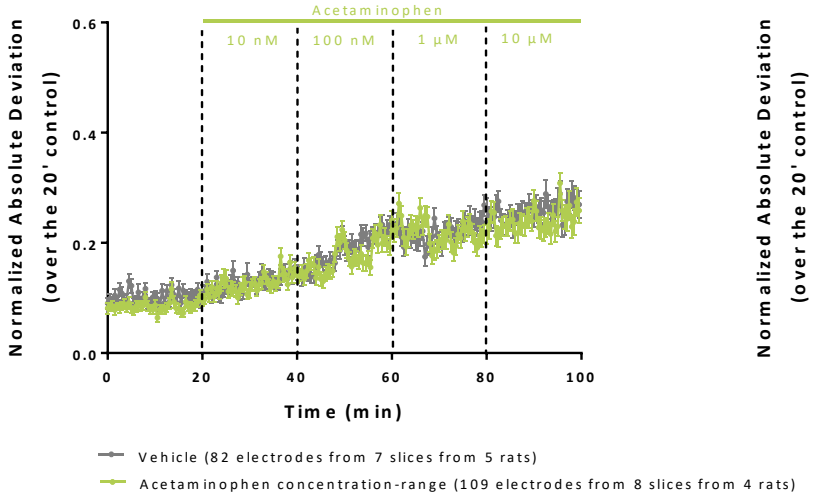
Cyclooxygenase

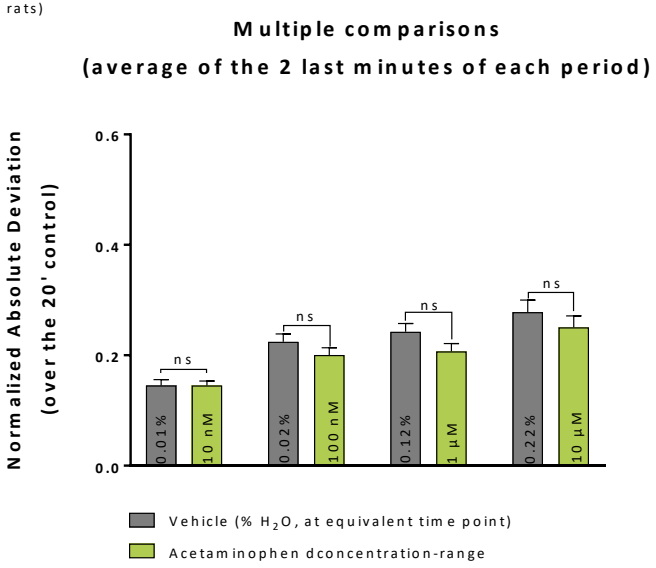
Main summary
PAG summary

Acetaminophen









- Acetaminophen, a cyclooxygenase inhibitor, did not modified the firing rate in comparison with vehicle.

THALAMIC RETICULAR NUCLEUS

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SUMMARY - Thalamic reticular nucleus

Main summary

Thalamic reticular nucleus

- Information about the thalamic reticular nucleus
- Materials & Methods
- Spontaneous and evoked firing activity

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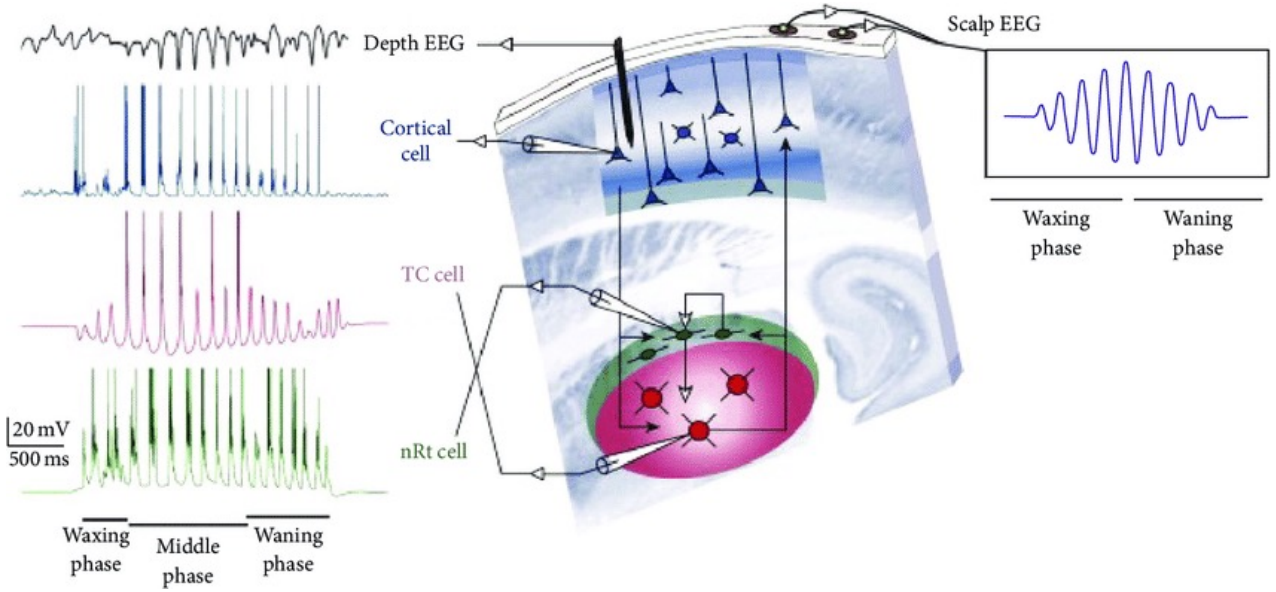
INTRODUCTION - Thalamic reticular nucleus

Main summary
TRN summary

The thalamus is able to generate transient oscillations occurring periodically during the early stages of slow-wave sleep. Given the extensive connectivity between cortical and thalamic neurons, the oscillations spread to the cortex from the thalamus. These oscillations (7-16 Hz), also named sleep spindles, are one of the rhythms that occur during the non-REM sleep.

Spindles can also be recorded *in vitro*, from acute brain slices. Spindles can occur spontaneously in the thalamic reticular nucleus (RTN) and in the thalamic ventrobasal (VB) nucleus. Spindles can also be elicited by a decrease in the extracellular magnesium concentration or an electrical stimulation of the internal capsule.



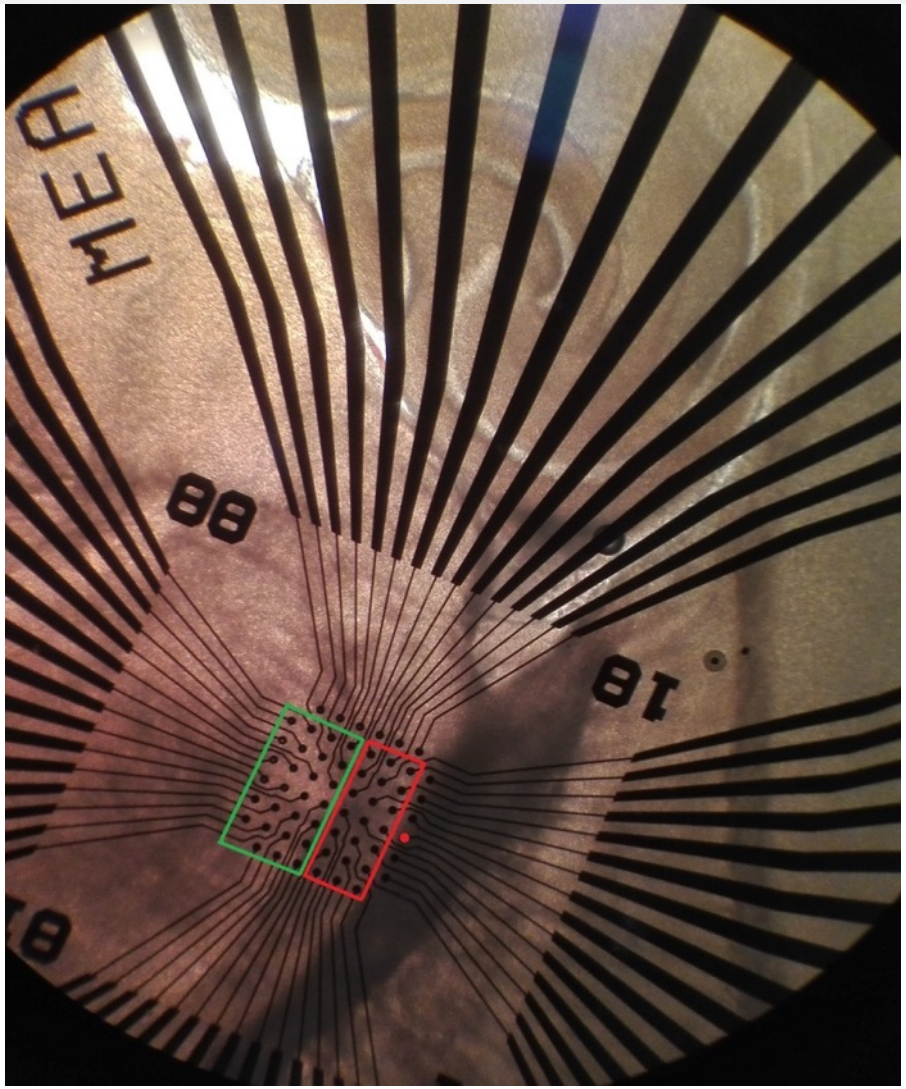
Spindles are generated in thalamocortical (TC) loop. The reticular (nRt) cells encounter the TC cells confined within the thalamus. The nRt cells inhibit TC cells which project excitatory inputs to the cortical cells. Cortical cells send excitatory input back to thalamic neurons. Sleep spindles arise from a cascade of recurrent, inhibitory, and excitatory signals between nRt, TC, and cortical cells

Sleep Spindles as an Electrographic Element: Description and Automatic Detection Methods. Coppieters et al., 2016

MATERIALS & METHODS - Thalamic reticular nucleus

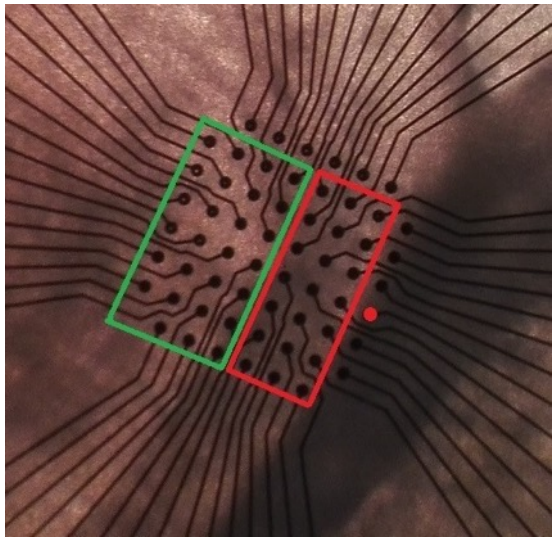
Main summary
TRN summary

Area of recording – horizontal rat brain slices



Horizontal slice from a 2 week-old Sprague Dawley rat

- Red point: example of electrode chosen to stimulate at the border between internal capsule (IC) and the thalamic reticular nucleus (RTN).
- Red square: electrodes located in the RTN region.
- Green square: electrodes located in the thalamic ventrobasal nucleus (VB).

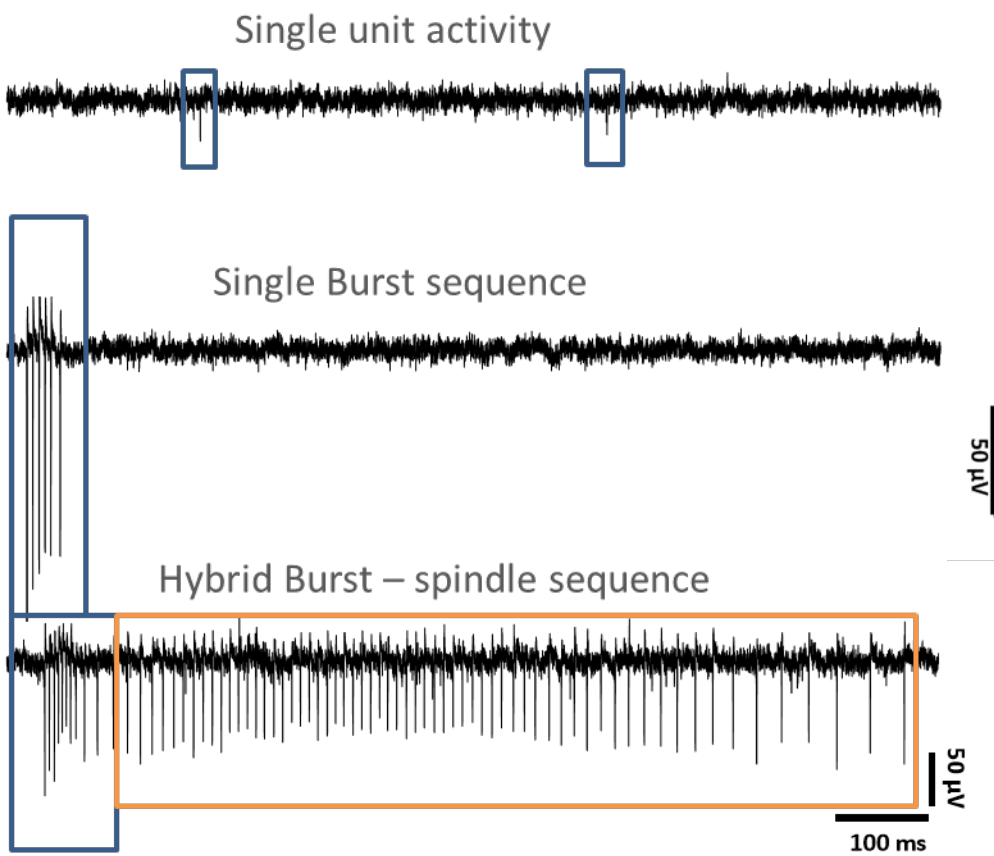


RESULTS - Thalamic reticular nucleus

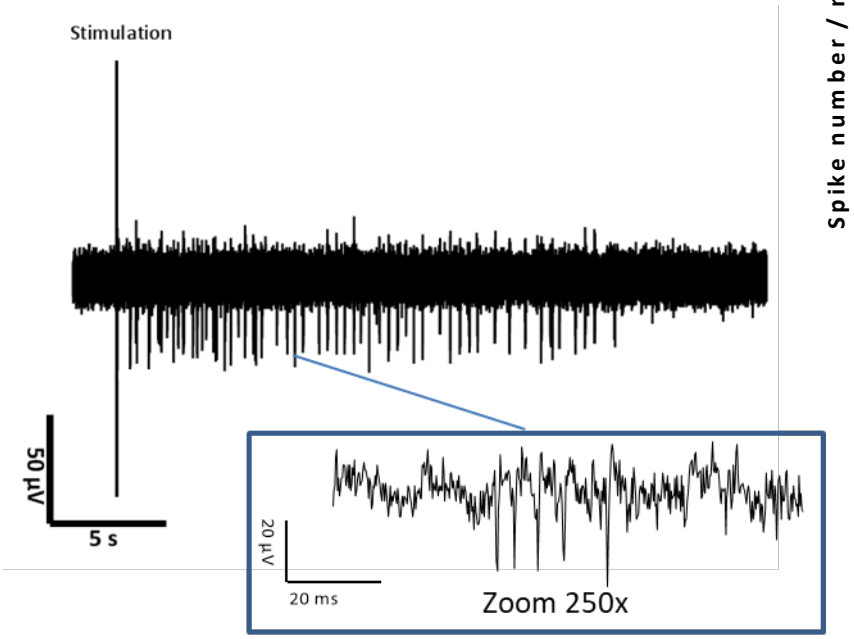
Examples of firing activity (Neuroservice data)

Main summary
TRN summary

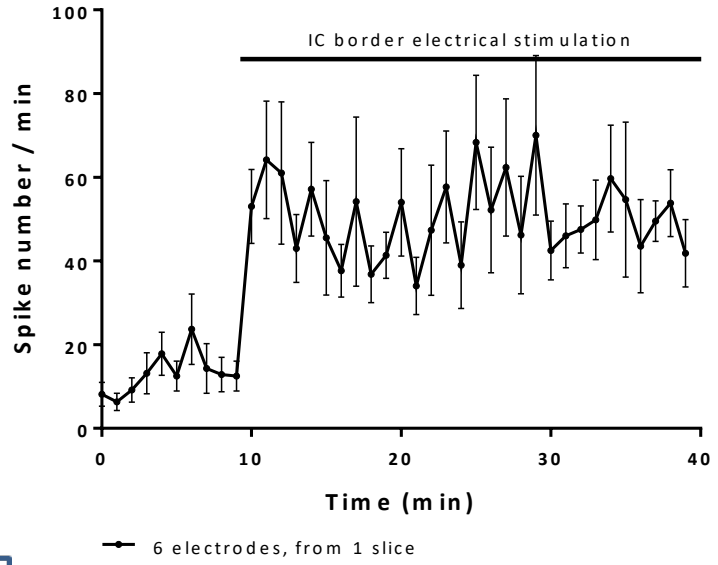
Spontaneous activity



Evoked Spontaneous activity



Above an example of evoked spindles recorded in the RTN after electrical stimulation of the internal capsule. Stimulation consisted in a single pulse (monopolar biphasic current pulse negative for 60 µs, then positive for 60 µs) applied at 60 s intervals.



The graph above represent the spike number detected as a function of time (1 min bin). Following 10-minute period of recording in absence of electrical stimuli, evoked spontaneous activity were triggered by stimulations applied every minute at the border of the IC, over a 30-minute period.

Contact us

Raymond Price, PhD, EMBA
Chief Business Officer
Raymond.price@neuroservices-alliance.com
Mobile - +1 (858) 649 9403

Jeffrey Hubbard, PhD
CSO, Brain Slice Electrophysiology
Jeffrey.hubbard@neuroservices-alliance.com
Mobile - +33 442 991 220