Super-resolution simultaneous visualization of diverse molecular species, such as proteins and mRNAs, is still challenging. In this work, we demonstrate the super-resolution simultaneous imaging of both proteins and mRNAs via expansion microscopy (ExM). To achieve it, we modify the protocol of expansion microscopy to better conserve the nanoscale details of both molecular species during the expansion process. We believe that this work will provide better understanding of molecular information of the brain and human body.

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P05.19

Aberration corrected inclined light sheet microscopy for high speed brain structure mapping

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Light sheet microscopy is a useful tool to obtain 3 dimensional images like confocal and multiphoton microscopy techniques. Using a very thin sheet of light with a uniform profile, light sheet microscopy differs in the way of depth sectioning from other methods that use either spatial gating or non-linear fluorescence excitation. This technique is superior to other techniques in terms of acquisition speed, photobleaching, and photoxicity, but degradation of image quality by aberration limits its usability to transparent specimens in specific geometries.

Adaptive optics (AO) can be utilized to compensate the optical aberration caused by spatial variance of refractive index within a specimen itself. Sensorless AO is one of the reliable techniques where endogenous guide stars can be used to obtain the distorted wavefront. For extended objects, various metrics can be applied to approximate and reconstruct an optimum correction map. Using sensorless AO, some low-order aberrations like defocus may be corrected by using electromechanical devices such as tunable lenses, but high-order aberrations which also degrade image quality cannot be overcome by simple physical adjustment of optic components.

Here we utilize a spatial light modulator (SLM) to realize correction of high-order Zernike modes and compensate specimen-induced optical aberrations in inclined light sheet geometries. We will see which metric is effective in correcting the optical aberration raised in light sheet microscopy and show diffraction limited resolution for tilted illumination and detection angles. Furthermore, we will propose the new configuration of sensorless AO where a single SLM is used to correct two distinct aberrations in both illumination and detection pathways. Our work will be meaningful to show the fact that light sheet microscopy could be used to image specimens in various geometries rather than the single orthogonal illumination, detection geometry in conventional light sheet microscopy.

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P05.20

Method of decellularized scaffold for tissue regeneration using tissue clearing

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Tissue engineering is based on biomaterials that can help restore organs. Various materials have been tested to restoring, maintaining and improving the functions of the body. Despite of limitation of massive production, decellularization is one of the best methods for making scaffold. However, among the various tissues in the human body, brain tissue is weaker and has higher cell density than other body tissues. When cells are removed by applying a general decellularization method, brain is easily to be damaged and get low reproducibility.

In this study, we confirmed that it is possible to preserve the intact form of brain and microarchitecture when the fixed brain tissues were decellularized with 4% hydrogel, especially polymerized in oil. The role of oil is prevention of forming excessive gel, therefore hydrogel was filled only in brain tissue. When decellularize with hydrogel brain scaffold, conserved tissue shapes and protein contents than without hydrogel.

Therefore, we suggest that this method can be widely used in various fields requiring tissue regeneration.

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P06.01

Drugs of abuse inhibit striatal dopamine transmission evoked by prefrontal cortex inputs

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Drugs of abuse target the reward system and have one common action in the brain: increasing dopamine (DA) in the striatum. Recently, it was shown that the activation of prefrontal cortex (PFC) inputs can evoke DA transients in the striatum, comparable to more conventional DA transients evoked by firing of midbrain DA neurons (DANs). In response to stimulation of glutamatergic inputs from the PFC, cholinergic interneurons fire action potentials and release acetylcholine (ACh), which in turns activate nicotinic ACh receptors on DA fibers and triggers DA release. It is yet unclear what is the function of this new form of DA transmission in vivo and the effects of drugs of abuse on it. Using in vitro voltammetry and transgenic mice with optogenetic techniques, DA transients were recorded in dorsal striatum by stimulating either PFC inputs or DAN fibers in the same brain slice. To our surprise, bath application of either cocaine, nicotine, ethanol, morphine, fentanyl, or THC all inhibited PFC-driven DA transients without affecting DAN-driven DA transients. Further experiments showed that while each drugs of abuse inhibit the PFC-driven DA transient, the mechanisms by which they do so differ. Furthermore, PFC-driven and DAN-driven DA transmission antagonized each other. When the stimulation

of PFC fibers preceded stimulation of DAN fibers, the DAN-driven DA transient was depressed, and vice versa. These antagonistic effects were significantly reduced in the presence of cocaine. These findings encourage a reconsideration of how DA transmission in the striatum is affected by drugs of abuse and also reveal a novel interaction between two mechanisms of DA release that may be compromised by drugs of abuse.

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P06.02

Cerebellar microcircuit regulates long-term fear memory by the STAT3-mediated excitatory-inhibitory balance

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It has been overlooked that emotional memory processing is associated with bodily movement particularly in fear memory consolidation and retrieval. Here, we found a novel mechanism by which input-output control of Purkinje cells *via* signal transducer and activator of transcription 3 (STAT3) might modulate long-term fear memory. Transcriptome analyses showed that the expression of glutamate receptor subunits, GluA1/2, were significantly increased in the Purkinje cell-specific STAT3-deficient mice compared to controls. The results demonstrate the critical role of STAT3 as a transcriptional repressor that modulates the expression level of glutamate receptors within the dynamic range. In the sense of fear memory processing, we discovered that long-term potentiation (LTP) was reframed to long-term depression at parallel fiber to Purkinje cell synapse in the STAT3 knockout mice. On the other hand, fear-conditioned Purkinje cells induced LTP at molecular layer interneuron to Purkinje cell synapse without being affected by STAT3. Unlike the synaptic changes, cerebellar STAT3 is independent on intrinsic excitability of Purkinje cells. Interestingly, STAT3-deficient mice had an aberrant long-term memory of fear, while no motor-related behavioral phenomenon was observed. All things considered, our data strongly suggest that the disruption of excitation-inhibition balance mediated by STAT3 in cerebellar microcircuits causes the aberrant fear memory formation, and might lead to develop the novel therapeutics for psychiatric disorders, such as posttraumatic stress disorders.

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P06.03

Slow presynaptic calcium dynamics gate long-lasting asynchronous release at the hippocampal mossy fiber to CA3 pyramidal cell synapse

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Action potentials trigger two modes of neurotransmitter release, with a fast synchronous component and a temporally delayed asynchronous release. Asynchronous release contribute to information transfer at synapses, including at the hippocampal mossy fiber to CA3 pyramidal cell synapse where it controls the timing of postsynaptic firing. Here, we investigated how different patterns of presynaptic firing control asynchronous release.

We find that asynchronous release at MF-CA3 synapses is biphasic and lasts on the order of seconds following repetitive stimulation. While the first phase is limited to a few hundred milliseconds and demonstrates a high release rate, the second phase lasts on the order of seconds and demonstrates a much lower release rate. Elevating the stimulation frequency or the external Ca^{2+} concentration increased the total rate of asynchronous release, but had no impact on the biphasic nature of asynchronous release, suggesting the dependency of asynchronous release on presynaptic Ca^{2+} dynamics. Direct MFBs Ca^{2+} imaging revealed slow Ca^{2+} decay kinetics and that the peak amplitude of Ca^{2+} transients was invariant during trains of action potentials. Last, we observed that asynchronous release was preferentially mediated by P/Q-type voltage-gated Ca^{2+} channels and that increasing presynaptic Ca^{2+} buffering with EGTA-AM selectively reduced the rate but lengthened the total asynchronous release.

Overall, our results reveal the main determinants of a slow and biphasic asynchronous release at MF-CA3 synapses. This long-lasting asynchronous release is well-positioned to influence CA3 pyramidal cell firing seconds following termination of granule cell bursting.

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P06.04

Action potential mediated long range cell to cell connections within rat suprachiasmatic nucleus (SCN): Exploring the characteristics of connections and morphology by using the custom-built optogenetic mapping system

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Normal neurons have stochastic behavioral action potentials, but neurons in the suprachiasmatic nucleus (SCN) have periodic spontaneous action potentials whose rates are changing over circadian time. In addition, as the central clock of mammals, the SCN clock cells constitute the nonlinear oscillating network and control the circadian rhythm with the characteristics of adaptation to strong synchronization and external light stimuli. As clock cells are smaller in size and their nucleus area than other neurons, it is not easy to study the anatomical structure and network connectivity. So there is little research on it. We explored the connection shape and characteristics of clock cells using a custom-built optical stimulus mapping system and protocol with the ChR2 transfected organotypic SCN slices. In order to generate coherent circadian rhythm, networks of clock cells have long - range inhibitory cell-to-cell connections that directly affect phase locking and synchronization. The long-range cell to cell connection we observed was an inhibitory connection via GABAa that could be blocked by Bicuculline, and an AP-mediated connection that could be completely eliminated by TTX. The connection that a given target clock cell can have is a sparse connection using the DBSCAN clustering method based on two experimental values obtained from the experiments: the physical distance and the difference in amplitude of the current measured in response to the optical stimulus of a given target cell, respectively. Thus, we suggest that the long range cell-to-cell connection in the SCN is an inhibitory AP-mediated connection through GABAa, and the number of independent

Drugs of abuse inhibit striatal dopamine transmission evoked by prefrontal cortex inputs

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Introduction

According to the conventional view, dopamine (DA) is released in the striatum in response to action potential firing that originates in DA neurons (DANs) in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc). Recently, it has been shown that synchronized firing of cholinergic interneurons (CINs) within the striatum can evoke DA release through activation of nicotinic acetylcholine receptors (nAChRs) (Cachope et al., 2012; Threlfell et al., 2012), which are expressed in striatal DA axons and presynaptic terminals (Sulzer et al., 2016). It was also shown that activation of thalamic and cortical inputs to CINs can induce DA release in the striatum via this mechanism (Kosillo et al., 2016; Threlfell et al., 2012). In this project, we have characterized basic properties of prefrontal cortex (PFC)-driven DA transmission in the striatum and compared with the properties of DAN-driven DA transmission. With this information, we will be better able to speculate on the type of mechanisms and processing that each mechanism is involved in vivo.

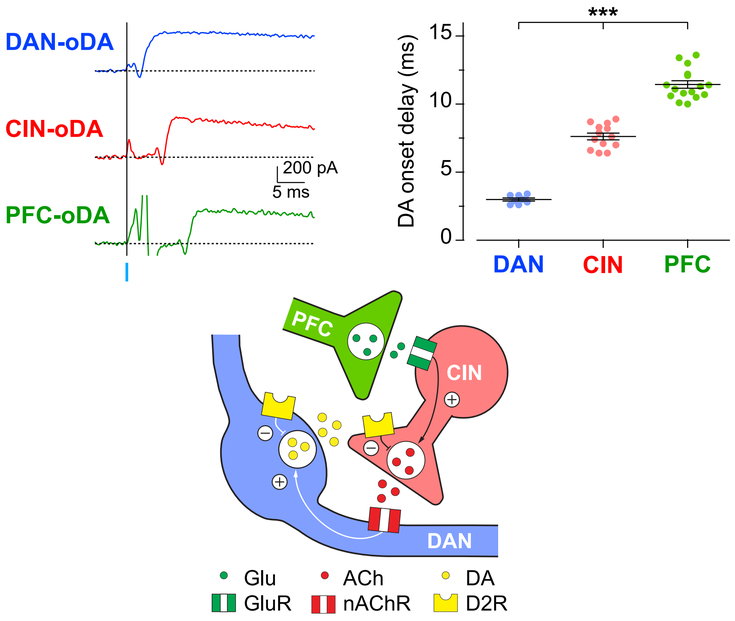
Methods

Recombinant adeno-associated virus (AAV) vectors were bilaterally injected to express opsins in different regions of mouse brain as below:

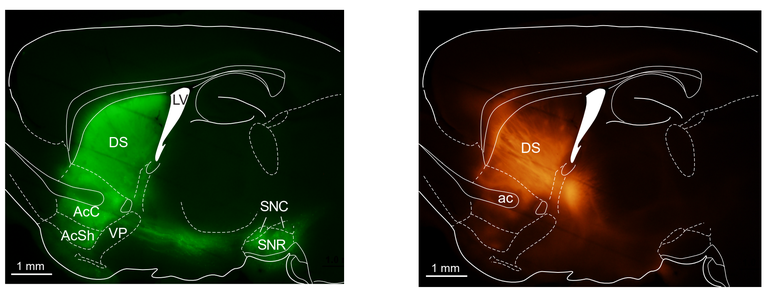
Mouse strain	Vector	Coordinates
DAT$^{IRES\text{-}cre+}$	AAV5-EF1a-DIO-hChR2(H134R)-EYFP	-3.3, +/-0.6, -4.5
ChAT$^{IRES\text{-}cre+}$	AAV5-EF1a-DIO-hChR2(H134R)-EYFP	+1.1, +/-1.2, -3.0
C57BL/6J	AAV5-CaMKII-hChR2(H134R)-EYFP	+2.1, +/-0.4, -2.3
C57BL/6J	AAV5-Syn-ChrimsonR-tdTomato	+2.1, +/-0.4, -2.3

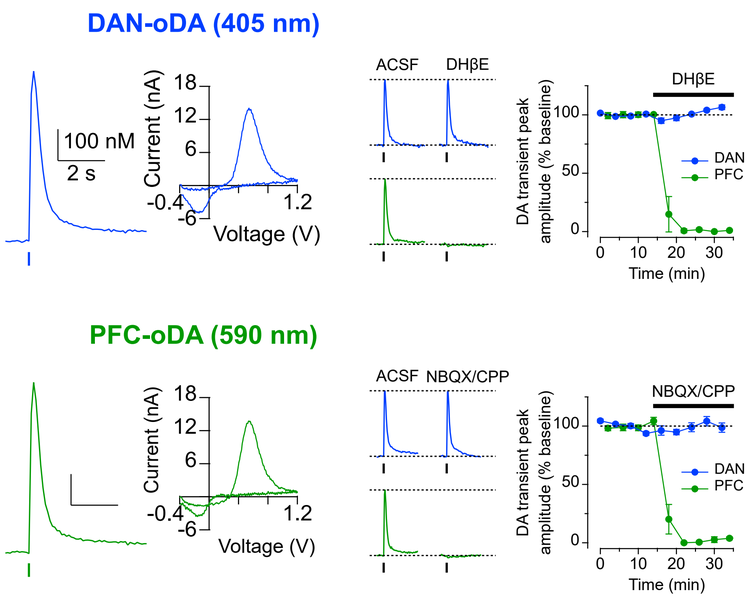
Brains slices in sagittal orientation (240 µm) were prepared using a vibratome (Leica) in an ice-cold cutting solution containing (in mM) 225 sucrose, 13.9 NaCl, 26.2 $NaHCO_3$, 1 NaH_2PO_4, 1.25 glucose, 2.5 KCl, 0.1 $CaCl_2$, 4.9 $MgCl_2$, and 3 kynurenic acid, and were recovered for 20 min at 33°C in artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 1 NaH_2PO_4, 2.5 KCl, 1.3 $MgCl_2$, 2.5 $CaCl_2$, 20 glucose, 26.2 $NaHCO_3$, and 0.4 ascorbic acid and maintained in the dark at room temperature prior recordings.

The onset of PFC-oDA is delayed compared to DAN-oDA.

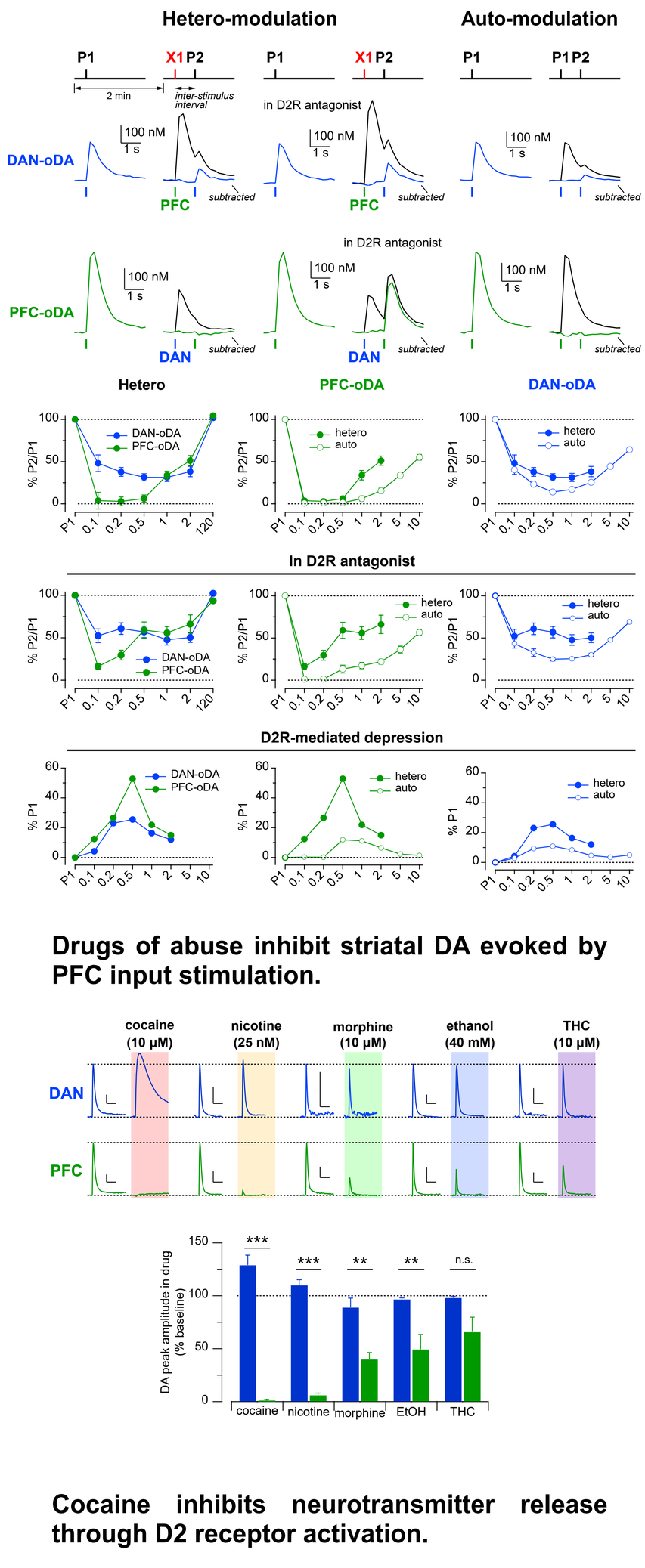


Dual optogenetic stimulation using DAT$^{ires\text{-}cre+}$ mouse injected with DIO-ChR2 in VTA and Chrimson in PFC.



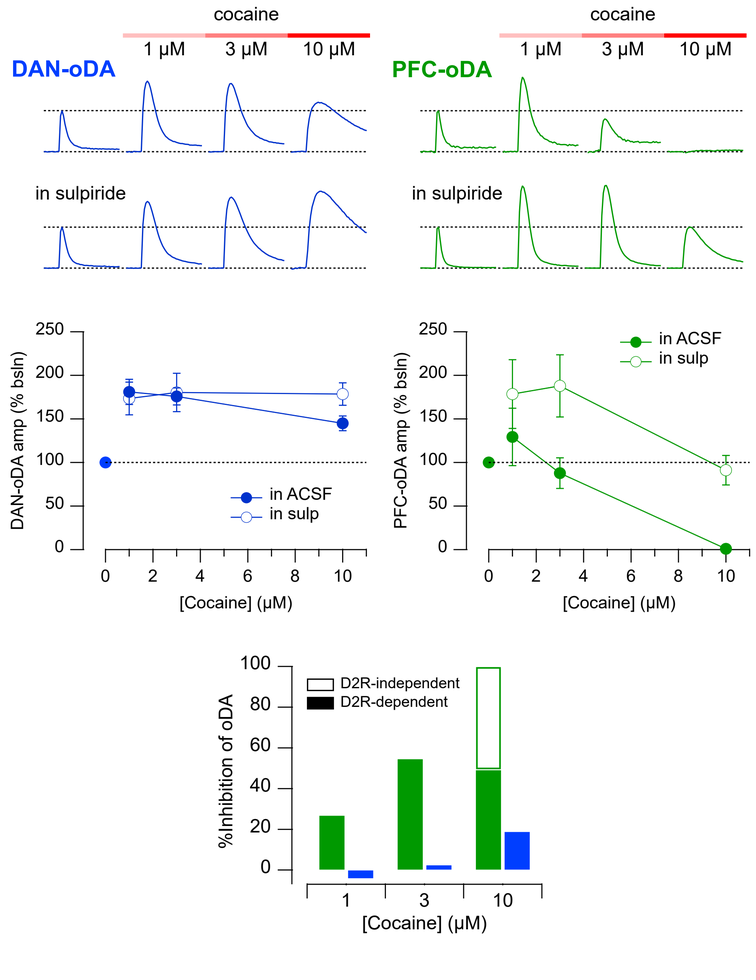


PFC signals can inhibit midbrain DA signals (and vice versa) when they precede.

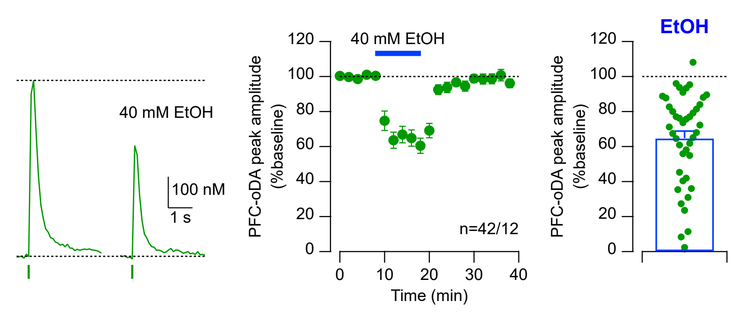


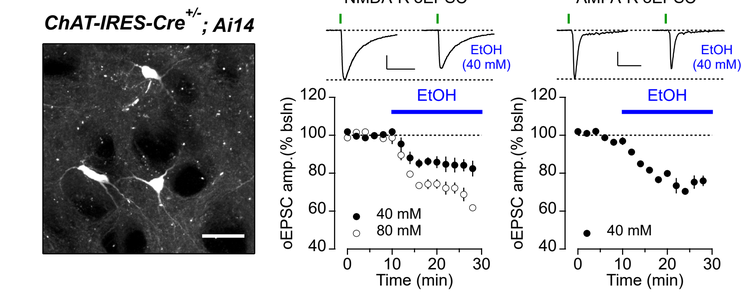
Drugs of abuse inhibit striatal DA evoked by PFC input stimulation.

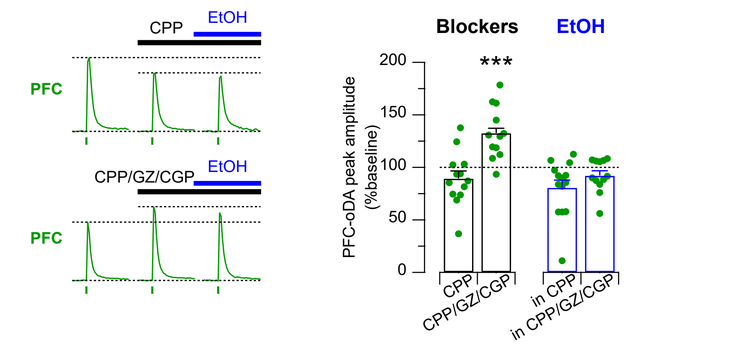
Cocaine inhibits neurotransmitter release through D2 receptor activation.



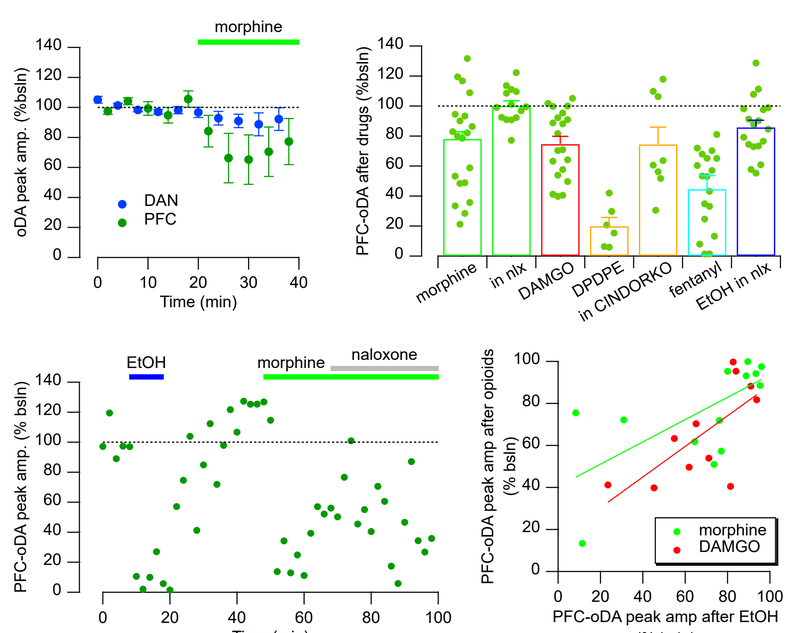
Ethanol inhibits NMDA-R and facilitates GABA-R activation.



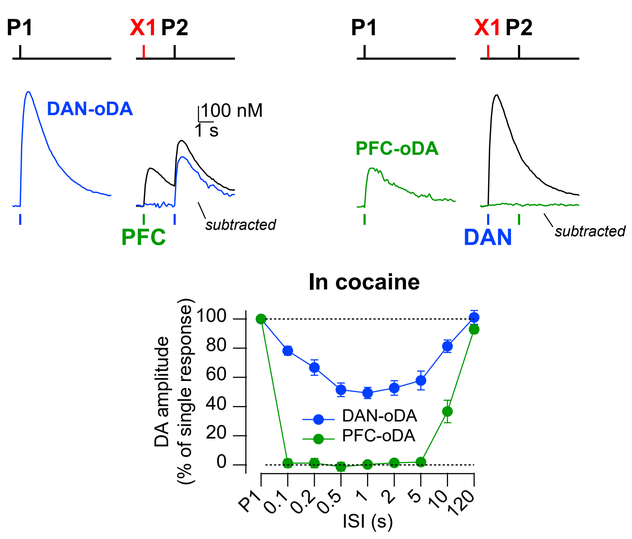




Morphine depresses PFC-oDA by activating μ-opioid receptors.



In the presence of cocaine, preceding midbrain DA signals shuts down PFC DA signals.



Summary

Using *in vitro* voltammetry with optogenetic techniques, DA transients were recorded in dorsal striatum by stimulating either PFC inputs or DAN fibers in the same brain slice.

PFC signals can inhibit midbrain DA signals (and vice versa) when they precede it.

PFC-evoked DA is inhibited by drugs of abuse via diverse targets.

Depression of PFC-oDA by preceding midbrain DA signals is further enhanced in the presence of cocaine.