Orexin/Hypocretin enhances synaptic strength in VTA dopamine neurons

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Orexin/Hypocretin and Reward

- Orexin/Hypocretin increases VTA neuron firing (Korotkova et al., 2003)
- Intra-VTA orexin/hypocretin increases dopamine in the Nucleus Accumbens (Narita et al., 2006) or PFC (Vittoz and Berridge, 2006)
- Orexin/Hypocretin neurons are activated when rats prefer morphine in a CPP paradigm which is blocked by intra VTA hypocretin antagonist (Harris et al., 2005)
- CPP for morphine is abolished in orexin/hypocretin peptide knockout mice (Narita et al., 2006).
- Orexin/hypocretin i.c.v. reinstates cocaine seeking (Boutrel et al., 2006)
How does orexin/hypocretin mediate the rewarding effects of drugs?

Can ox/hcrt cause synaptic plasticity in dopamine neurons?
Why is synaptic plasticity in the VTA important?

- Glutamatergic synaptic plasticity plays a key role in neural plasticity relevant to addiction
  - Induction of behavioral sensitization is dependent on activation of NMDA receptors in the VTA (Kalivas and Alesdatter, 1993)
- Synaptic plasticity of dopamine neurons in the VTA may play a key role in the reinforcement of reward.
Patch Clamp Recording from VTA neurons

AMPA EPSC
-70 mV

NMDA EPSC
+40 mV

Currents

(Cortical afferent) → (VTA Dopamine neuron) → (Record EPSC)

Stim. → (Record EPSC) → (Excitatory Postsynaptic Currents)
OxA/Hcrt1 concentration-dependently increases NMDAR EPSCs in VTA neurons

n=12

NMDA Amplitude (% increase)
OxA/Hcrt1 does not potentiate AMPA EPSCs in VTA neurons

n=8
Orexin/Hypocretin Pharmacology

Pre-pro ox/hcrt

OxA/Hcrt1

OXR1/Hcrt1R

G_q

OXB/Hcrt2

OXR2/Hcrt2R

G_q

G_i/o
OxA/Hcrt1 mediated potentiation of NMDAR EPSCs is inhibited by OX1/hcrt1 receptor antagonist

n=8
GLU
NMDAR
NR1
NR2A/?
AMPA
PFC
PPN
LH-hcrt neuron
OXA
OXA
OXA
OXA
OXA
OXR1
+ 
PLC/PKC
NR1
NR2A/?
1
2
VTA Neuron
OxA/Hcrt1 on AMPAR synaptic transmission

- Activation of NMDARs is an important component for VTA long term potentiation (LTP)
- AMPAR/NMDAR is measure of LTP
NMDAR activation precedes AMPAR plasticity

OxA/Hcrt1, CRF, cocaine
NMDAR activation precedes AMPAR plasticity

Stress, cocaine, other drugs of abuse

Does orexin A potentiation of NMDARs facilitate cocaine-mediated AMPAR plasticity?
**OXR1/Hcrt1R antagonist blocks cocaine induced potentiation of AMPAR/NMDAR ratio**

5 days cocaine or saline +/- OXR1 antagonist
OXR1/Hcrt1R antagonist blocks cocaine induced potentiation of AMPAR/NMDAR ratio

5 days cocaine or saline +/- OXR1 antagonist
Does OxA/Hcrt1 increase AMPA/NMDA ratio?

Slices are incubated with OxA/Hcrt1 for 5 minutes.
OxA/Hcrt1 increases AMPAR/NMDAR hours after application

**AMPAR/NMDAR Ratio**

- **control**: n=8
- **15 min**: n=8
- **3-4 hours**: n=8

* denotes p < 0.05
** denotes p < 0.01
OxA/Hcrt1 causes a late phase increase in AMPAR mediated synaptic transmission.
OxA/Hcrt1 causes a late phase increase in AMPAR mediated synaptic transmission that is NMDAR dependent.
**Does OxA/Hcrt1 mediated plasticity of dopamine neurons have behavioral consequences?**

- Activation of NMDARs is an important component for VTA LTP (Bonci & Malenka, 1999) and the development of cocaine sensitization (Kalivas & Alesdatter, 1993)

- Behavioral sensitization is a progressive increase in locomotor response to the same cocaine dose.

- Since cocaine sensitization is dependent on NMDAR activation in the VTA, we hypothesized that OxA/Hcrt1 may have a role in behavioral sensitization to cocaine.
OXR1/Hcrt1R antagonist blocks cocaine sensitization

n=13

OXR1/Hcrt1R antagonist blocks cocaine sensitization

n=13
Behavioral sensitization is blocked with intra-VTA injections of OXR1/Hcrt1R antagonist
Hypothesis

Orexin/hypocretin has a profound role in altering synaptic plasticity in a neural circuit important for motivation.

Does orexin/hypocretin signaling mediate motivated behavior?

ie. if orexin/hypocretin receptors are blocked, will rats work as much to get cocaine?
Self-administration Progressive Ratio

0.5 mg/infusion cocaine
Paired with tone and light

0.0 2.5 5.0 7.5 10.0 12.5 15.0

0 50 100 150 200

Lever Presses

Progressive ratio

Surgery  FR1  FR3  FR5

Naive  Vehicle  Vehicle  SB 334867
Vehicle treated rats do not reduce pressing for the duration of the experiment.
OXR1/Hcrt1 antagonist reduces “motivation” in progressive ratio test in cocaine self-administering rats

SB 334867 10 mg/kg

n=12
Cumulative response shows the pattern of presses for cocaine in vehicle and SB334867 treated rats.
Food self administration

$n=9$
Orexin/Hcrt 1 receptor signaling is not involved in motivation for food

SB 334867 10 mg/kg

n=10
Orexin/Hcrt 1 receptor signaling is not involved in motivation for food

SB 334867 20 mg/kg

n=12
Cumulative response shows the pattern of presses for food in vehicle and SB334867 treated rats.
Cocaine Self-Administration increases OxA/Hcrt1 potentiation of NMDARs

Food

Cocaine

n=8
OxA/Hcrt 1 mediated potentiation of NMDAR is not different between food restricted (sham) and naïve rats

**Naive**

![Graph showing NMDAR eEPSC (% Baseline) over time for Naive group.

**Sham**

![Graph showing NMDAR eEPSC (% Baseline) over time for Sham group.]

n=7  n=9
Cocaine self-administration potentiates OxA/Hcrt1-mediated synaptic plasticity in the VTA
Summary

- OxA/Hcrt1 potentiates NMDA currents in DA neurons of the VTA.
  - OxA/Hcrt1 enhance:
    - synaptic strength in the mesolimbic system
    - burst firing of DA neurons, and increase in DA release.

- OxA/Hcrt1 causes a late phase increase in AMPAR mediated synaptic transmission
  - Facilitating dopamine’s role in reinforcement?
Summary -2

- OXR1/Hcrt1R antagonist blocks cocaine sensitization, indicating that activation of orexin/hypocretin 1 receptors in the VTA is required for the development of sensitization.

- Orexin/hypocretin signaling is involved in “motivation” for cocaine but not food seeking.

- Cocaine self-administration potentiates orexin/hypocretin-mediated plasticity in the VTA.
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Prolonged application causes a long-lasting increase in NMDAR EPSCs

n=6
Does OxA/Hcrt1 potentiate NMDARs in dopamine neurons?
OxA/Hcrt1 increases NMDAR EPSCs in VTA dopaminergic neurons
Orexin plays a gatekeeper role in that it enables neuroplasticity in excitatory synapses in the VTA.

1. Ox/hcrt potentiation of NMDA promotes burst firing and increases DA release.

2. Ox/hcrt late phase potentiation of AMPARs may prolong burst firing.

This plasticity may underlie the intensification of goal-directed behavior.
OXR1 antagonist reduces food self-administration in the presence of cocaine

n=12
OXR1 antagonist reduces breakpoint in the presence of cocaine

n=12
Dopamine is required for the orexin-mediated reduction in food seeking

![Graph showing the number of presses and reinforcers across different conditions. The graph indicates that dopamine is required for the orexin-mediated reduction in food seeking.](image)
Dopamine is required for the orexin-mediated reduction in food seeking
SB 334867 attenuates potentiation of breakpoint by a single injection of cocaine.
**OXR1 signaling needed for cocaine PR but not food PR**

- Is increased dopamine required for orexin release?
  - Can blocking orexin receptors in food treated rats reduce breakpoint after injection of GBR12909 (5 mg/kg) or cocaine (15 mg/kg)?

- Is orexin signaling involved only for highly motivational substances?
  - If you increase the reinforcing value of the reward, ie. Sucrose or high fat, will the orexin antagonist reduce seeking?

- Does cocaine potentiate orexin release/signaling?
SB 334867 (10 mg/kg) does not reduce motivation for sucrose

n=12
SB 334867 (20 mg/kg) does not reduce motivation for sucrose

n=9
**OXR1 signaling needed for cocaine PR but not food PR**

- Is increased dopamine required for orexin release?
  - Can blocking orexin receptors in food treated rats reduce breakpoint after injection of GBR12909 (5 mg/kg) or cocaine (15 mg/kg)?

- Is orexin signaling involved only for highly motivational substances?
  - If you increase the reinforcing value of the reward, ie. Sucrose or high fat, will the orexin antagonist reduce seeking?

- Does cocaine/dopamine potentiate orexin release/signaling?
  - Is there a change in pre-pro orexin in the LH?
  - Is there an alteration of orexin-mediated plasticity in the VTA?
Future experiments

- Are sucrose pellets not reinforcing enough? Is orexin signaling involved in motivation for high fat pellets?

- Does chronic cocaine change levels of pre-pro orexin or orexin A released?

- Is the OXR1 antagonist mediating the reduction in cocaine seeking acting in the VTA?
Orexin and Self-Administration

- Single injection (icv) of OxA induced persistent elevations of ICSS thresholds in drug naïve rats (Boutrel et al., 2006)

- OxA (icv) reinstated cocaine & food self admin (2 wk extinction) (Boutrel et al., 2006)

- OxA induced reinstatement was partially blocked by adrenergic and CRF antagonists (Boutrel et al., 2006)

- OXR1 antagonist (ip) blocked footshock induced reinstatement (Boutrel et al., 2006)

- OxA (icv) for 3 consecutive days did not alter cocaine self administration (Boutrel et al., SFN 2004)

- OxA (icv) did not alter progressive ratio for cocaine (0.25 mg/infusion; Boutrel et al., SFN 2004)