Multi-organ-on-chip Device for Modeling Opioid Reinforcement and Withdrawal, and the Negative Affective Component of Pain: a Therapeutic Screening Tool

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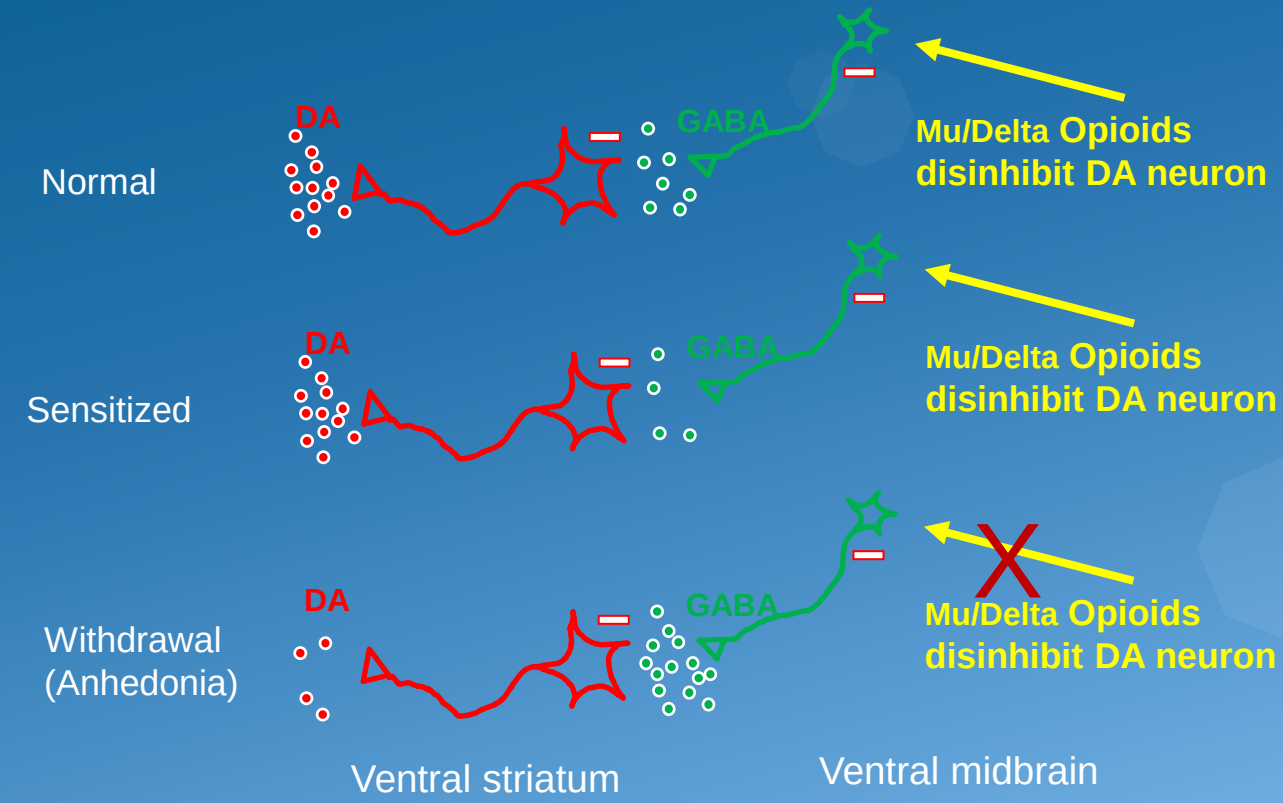
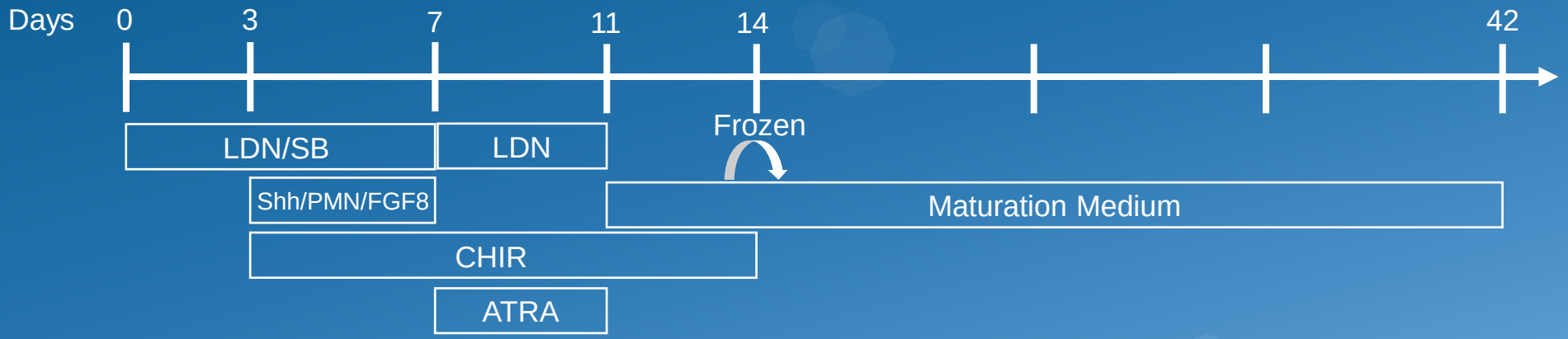
Objective

Design “multi-organ-on-chip” model systems based on human cells and use them to better understand the addictive process at a molecular level and to potentially identify new therapeutics to treat drug addiction and chronic pain.

How Opioids Affect Neurons



Midbrain Neuron Differentiation Protocol

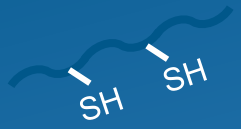


iPS Cell Line: Lothian Birth Cohort 1936 iPSC Collection, CS1185iCTR-LBCn2 (EDi044-A)

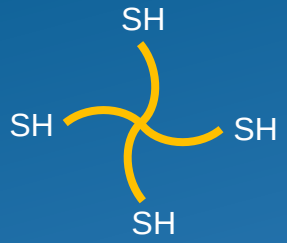
LDN: BMP inhibitor SB: TGF-β inhibitor Shh: Sonic Hedgehog PMN: Purmophamine FGF8: Fibroblast growth factor 8
CHIR: GSK3 inhibitor ATRA: All-*trans* retinoic acid
Maturation Medium: BDNF, GDNF, dbCAMP, Ascorbic acid, DAPT, TGF-β3

Protocol adapted from Laperle et al. Nat Med 2020

HA Hydrogel Fabrication Using Photocrosslinking



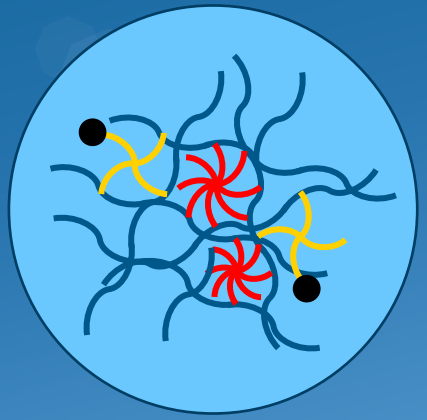
Thiolated HA



4-arm PEG-Thiol

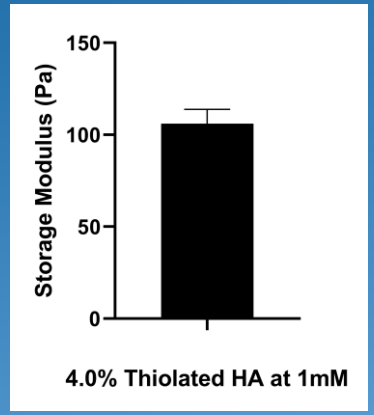
UV

LAP



Integrin-Binding Peptide

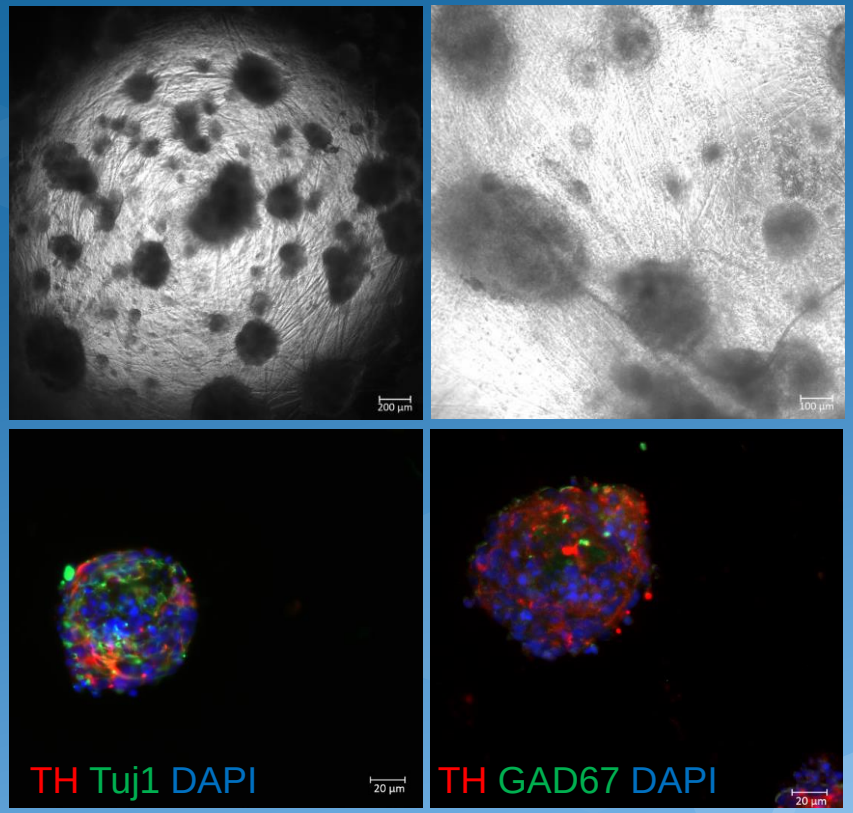
8-arm PEG Norbornene



Storage Modulus = 106.09 ± 19.34 Pa (N = 3, 13 sweeps from each gel)
HA: hyaluronic acid, ~700 kDa, 4% thiolation, 0.5wt%
Cysteine-terminated RGD peptides added to ~25% of PEG-thiol arms for cell adhesion.
LAP: type I photoinitiator lithium phenyl(2,4,6-trimethylbenzoyl)phosphinate, 0.025 wt%

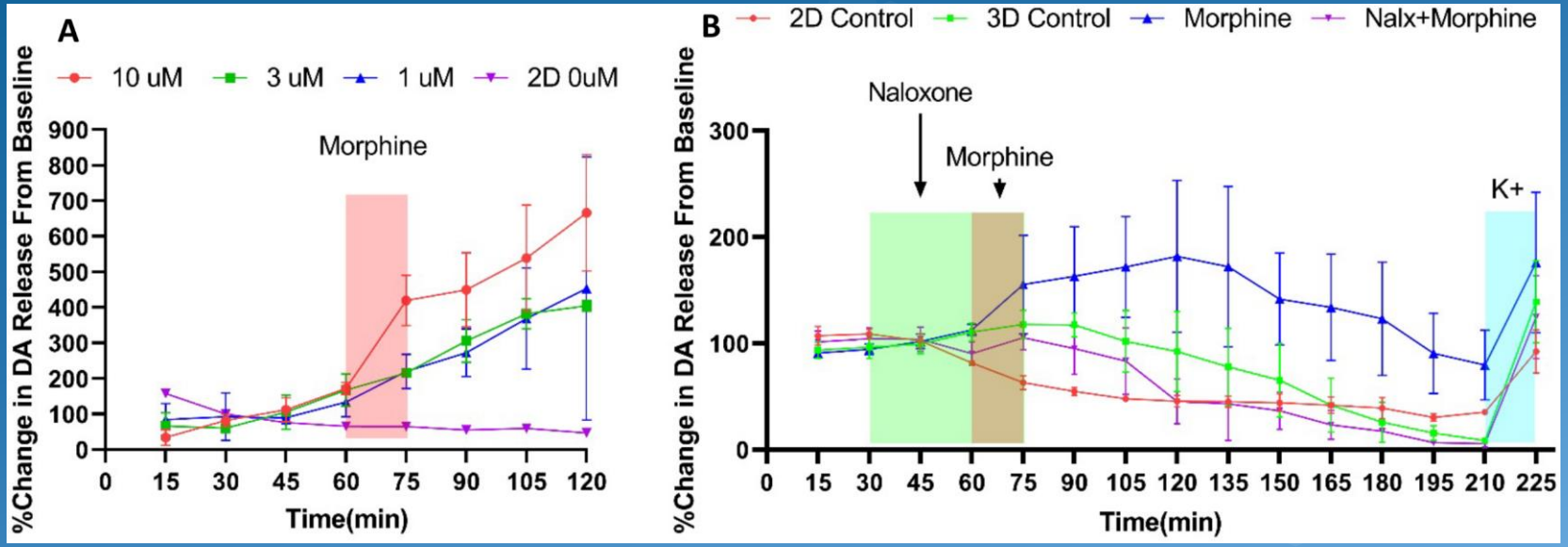
iPSC-derived Midbrain Populations in HA Hydrogel

- Network formation was observed
- Cryosections of the gel stained positive for midbrain population markers
  - Neuronal marker β-tubulin III (Tuj1)
  - Dopaminergic neuron marker tyrosine hydroxylase (TH)
  - GABAergic neuronal marker glutamate decarboxylase 67 (GAD67)



Line 1185

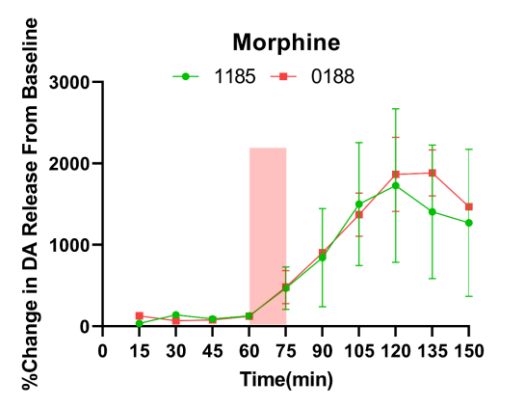
Dopamine Release Response to Morphine and Naloxone in 3D Culture

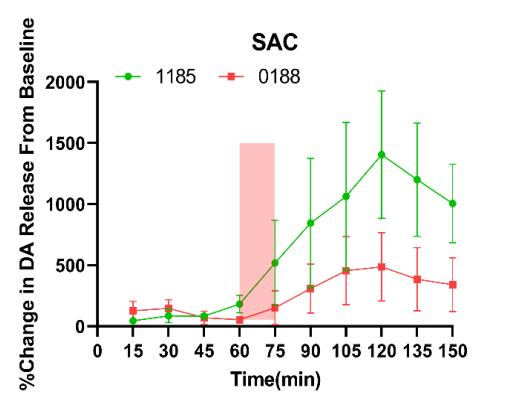


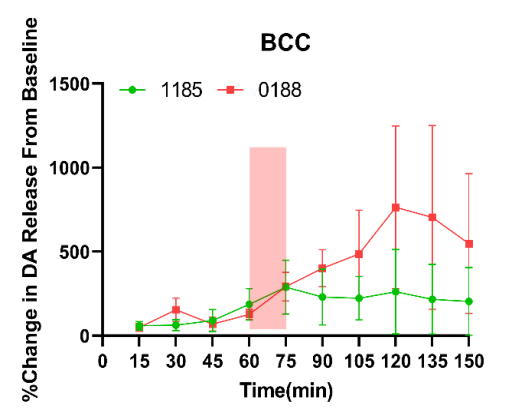
Line 0188
N = 3
Error Bar = Standard Deviation

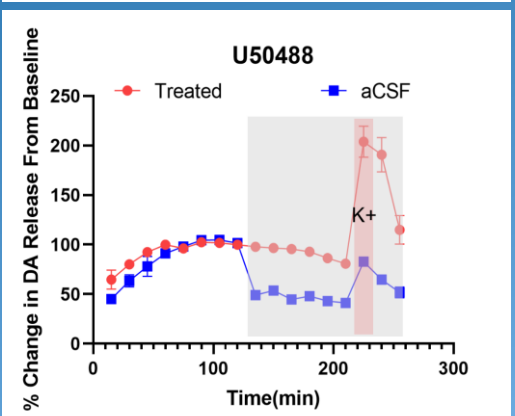
Line 0188
[Morphine] = 10µM; [Naloxone] = 30µM; [K+] = 100 mM
N = 3
Error Bar = Standard Deviation

Dopamine release induced by morphine, GABA antagonists, and κ-opioid agonist



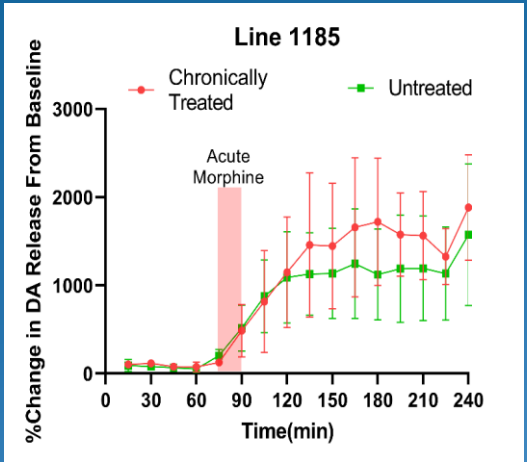


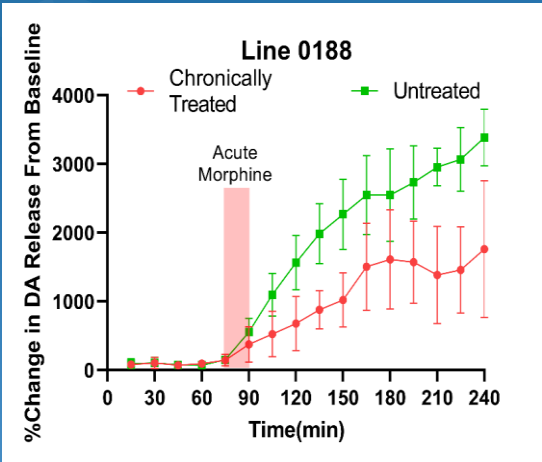




N = 3 – 5
[Morphine] = 10μM
[Bicuculline] = 20μM
[2-Hydroxy-Saclofen] =200μM
[U50488] = 10μM
[K+] = 100mM
Error bar = Standard Deviation

Effect of chronic morphine exposure followed by withdrawal on dopamine release induced by a subsequent morphine challenge

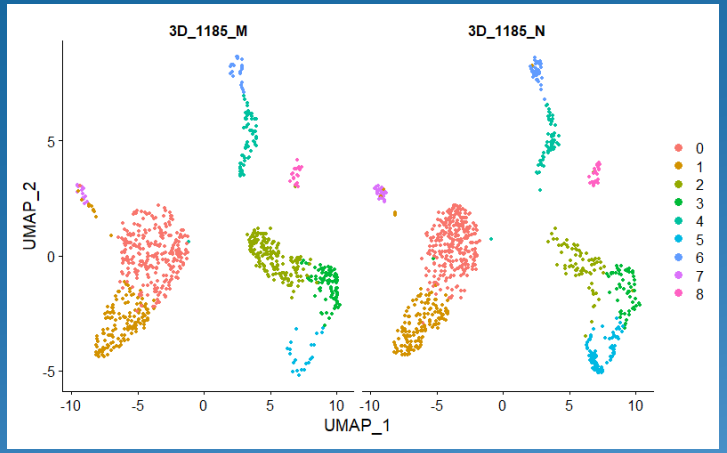


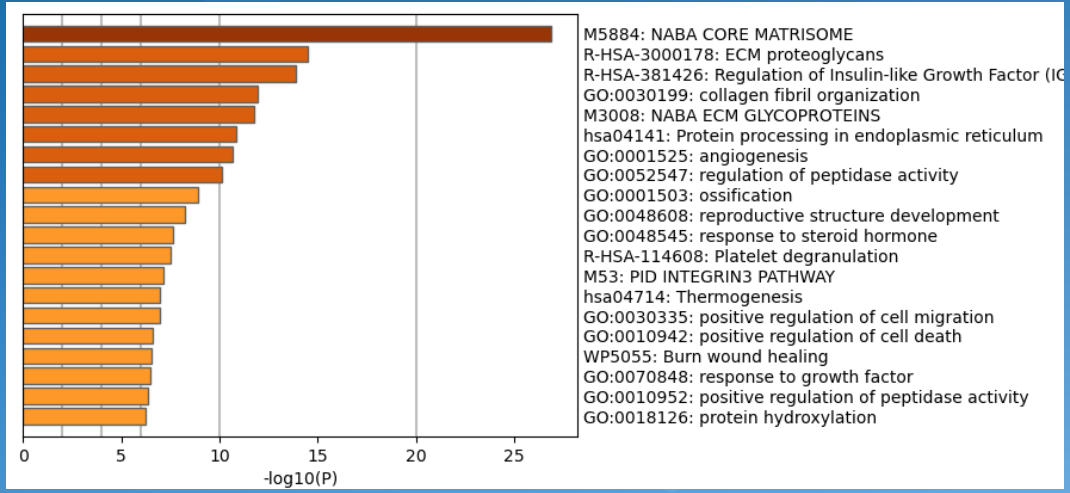


N = 4 - 5
[Morphine] = 10µM
Error bar = Standard Deviation

Chronic exposure: 4 h daily, 10 µM morphine, 7 days
Withdrawal: additional 7 days before acute morphine exposure

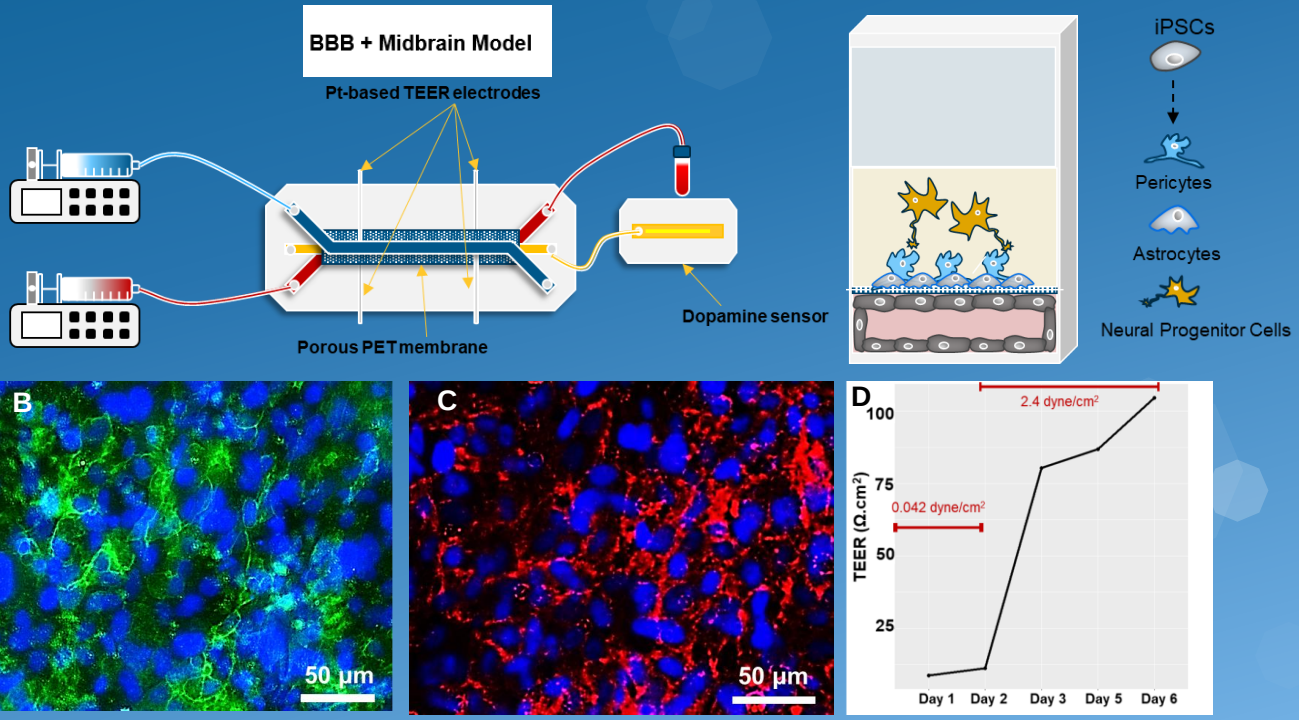
scRNAseq of Chronic Morphine Treatment



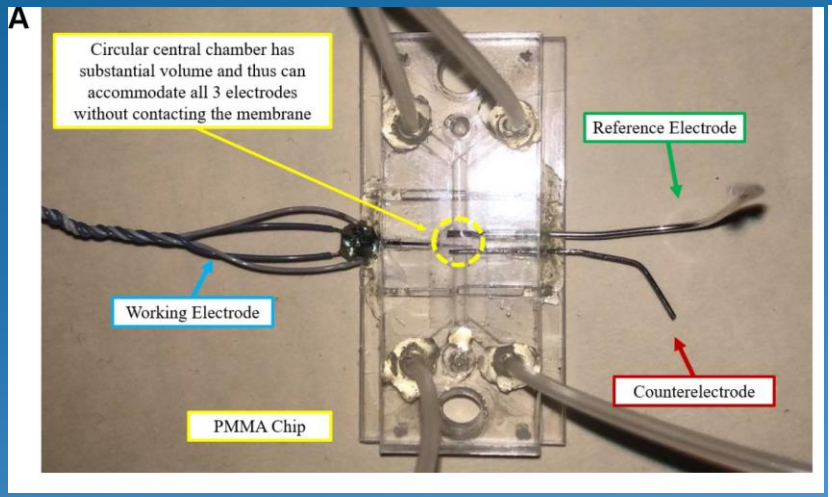


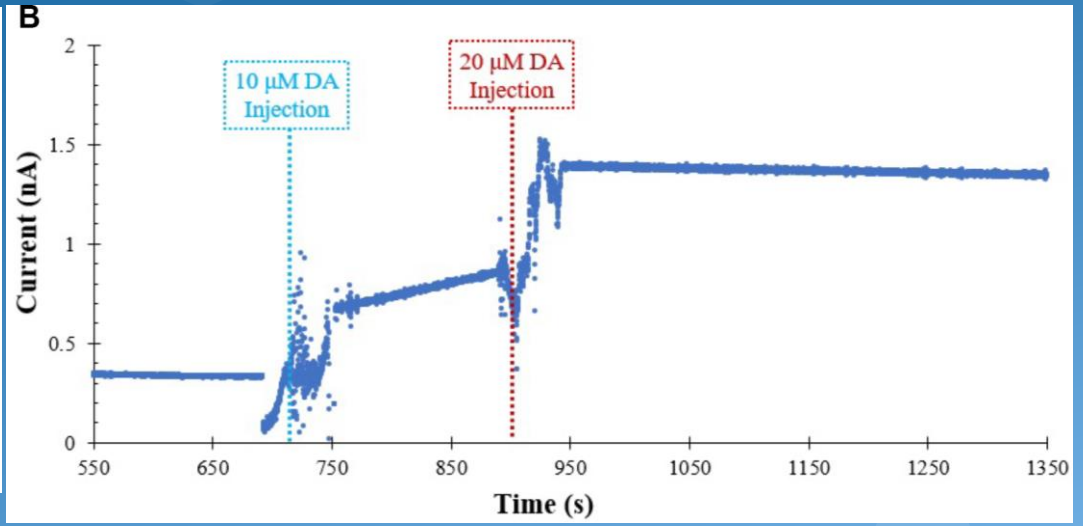
Cluster 2 Differentially Expressed Gene Ontology

Immortalized brain microvascular endothelial cells (hBMECs) cultured in the BBB chip



Integration of PMMA-based BBB chip with DA microarray sensor





Summary

- Observed reproducible, concentration-dependent, morphine-induced dopamine release from 2D and 3D cultures of two iPSC lines that is blocked by naloxone.
- Exposure to the GABA-B receptor antagonist, 8-OH-saclofen, increases basal efflux of dopamine indicating that dopamine neurons are tonically inhibited by the presence of endogenous GABA, reproducing the in vivo midbrain circuitry.
- Exposure to the kappa opioid receptor agonist, U50488, significantly attenuates basal and K+-induced dopamine efflux.
- Preliminary experiments have provided evidence of plasticity in dopamine release following chronic morphine exposure. The amplitude of the dopamine response to morphine challenge was attenuated in chronically-exposed cultures of line 0188 (male), but not line 1185 (female). Such tolerance in dopamine response potentially models the anhedonic component of withdrawal.
- Observed a substantial effect of shear stress on TEER and achieved values > 100 Ω.cm2.
- Incorporated on-line amperometric dopamine detection with silicon wafer-based electrodes into chip design.

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